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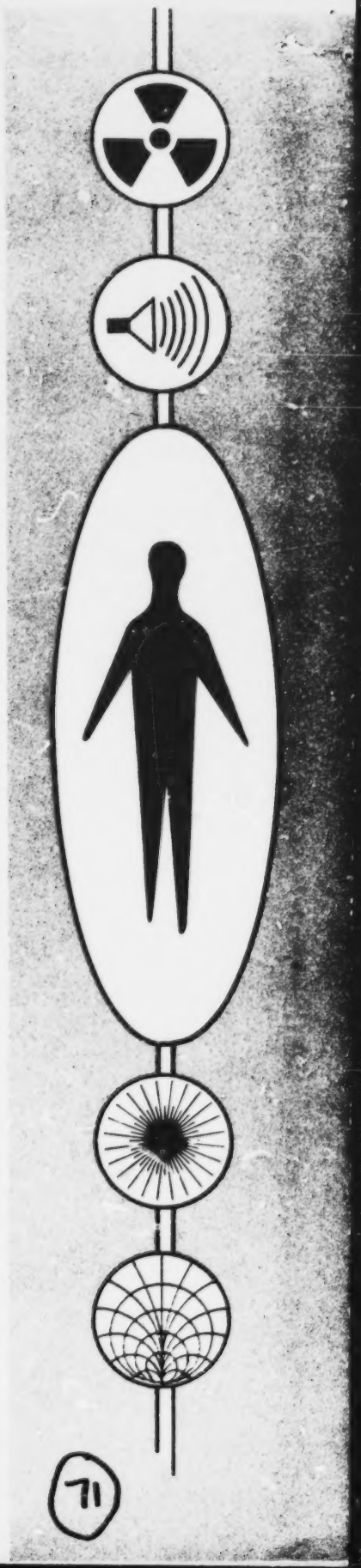
PHYSICAL MECHANISMS for BIOLOGICAL EFFECTS of ULTRASOUND



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PHYSICAL MECHANISMS for BIOLOGICAL EFFECTS of ULTRASOUND

BASED ON A SERIES OF LECTURES DELIVERED BY

Dr. Wesley L. Nyborg

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FOREWORD

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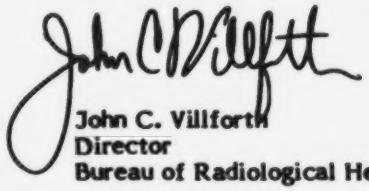

John C. Villforth
Director
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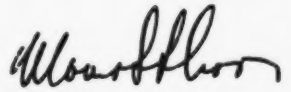
PREFACE

The Division of Biological Effects of the Bureau of Radiological Health, Food and Drug Administration, plans, conducts, and supports experimental and epidemiologic research on the biological effects of exposure to ionizing and nonionizing radiations. The increasing use of ultrasound in diagnostic and therapeutic medical procedures and our statutory responsibility under P.L. 90-602 mandate the evaluation of the possible risks that may be associated with the use of this modality. The results of research and evaluations supported and conducted by the Bureau's Division of Biological Effects facilitate the development of scientific and rational bases for regulation.

During spring, 1976, as a member of the Bureau of Radiological Health staff, Dr. Nyborg delivered a series of lectures on ultrasound biological effects and their underlying mechanisms. The lectures were jointly sponsored by the bureau and the School of Engineering Acoustics Program, Catholic University of America. This document was prepared by Dr. Nyborg on the basis of the lectures, so that the research results and a unified interpretation can be available to the scientific community.

The resulting report is a significant source of information on mechanisms by which ultrasound may produce changes in biological systems.



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ABSTRACT

In this report, available information is reviewed on the principles or mechanisms, as they are often called, by which ultrasound produces changes in living systems. In the science of biological ultrasound, as in any other science, a knowledge of principles is valuable in that it enhances our ability to make predictions. It is natural to stress physical mechanisms because ultrasound is a physical agent. The mechanism best understood is that of sonically generated heat. Temperature elevations can lead to significant alterations in rates of biochemical reactions, to structural breakdown of macromolecules and membranes, and to abnormalities in developing organisms.

Many experiments have been done in which ultrasound is applied to aqueous suspensions of macromolecules or cells; in these, enzymes may be inactivated or cells modified in structure and/or function. Here (when temperature elevation is avoided) the mechanism is usually sonic cavitation, an activity of small bodies comprised of gas or vapor.

When ultrasound produces bioeffects under conditions where temperature elevation is ruled out the mechanism can sometimes be identified as one associated with time-averaged sonically generated stresses. Among mechanisms in this class are the phenomena of radiation force (time-averaged force on a particle or a structure), radiation torque (time-averaged torque on a small body, which may cause it to twist or spin) and acoustic microstreaming (time-averaged circulations set up in fluid-filled spaces). Viscous stresses associated with acoustic microstreaming are especially important near microscopic gaseous bodies; it is important to know whether these exist in mammalian systems.

The report also contains a brief summary of available data for effects of ultrasound on mammalian tissues. In particular the lowest intensity levels at which significant effects have been reported are compared with levels generated by equipment presently being used in diagnostic medical practice. Attempts to explain these bioeffects data in terms of principles reveal the inadequacy of our understanding at this time. Continued and intensified research on mechanisms is obviously needed, especially for viscoelastic and inhomogeneous media similar to tissues. Until these mechanisms are known we cannot confidently extrapolate to clinical situations the results obtained from laboratory experiments.

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PHYSICAL MECHANISMS FOR BIOLOGICAL EFFECTS OF ULTRASOUND

Wesley L. Nyborg+

1. INTRODUCTION

In this report we treat biophysical principles involved in a situation where ultrasound* interacts with a biological system and, as a result, changes occur in the system. Many examples are known of such bioeffects; reviews are available (1-7). In some instances the sonic action is accompanied by temperature elevation, and the biological change is found to be just that which would have occurred if the same temperature elevation had been imposed on the system without sound. The action is then said to occur by a thermal mechanism. Much is known about sonic generation of heat, and also about heat transport; these topics are discussed briefly in Section 2.

Under other circumstances significant temperature elevation is prevented by cooling the system, by pulsing the ultrasound, or by other means. When the biological system is a suspension of bio-molecules or cells, the sonic action can then usually be attributed to some form of cavitation. This is a loosely defined term which is used to represent activity, in a sound field, of gas-filled bodies or bubbles, or of "cavities" which contain varying amounts of vapor or gas. In aqueous media a relatively violent form of cavitation may lead to production of the highly reactive free radicals H and OH, and hence to chemical changes mediated by them. Also significantly biologically are more gentle forms of sonic cavitation; mechanical stresses are developed near vibrating gas bubbles which can lead to structural changes in cells and macromolecules. In Section 3 we take up various aspects of sonically generated cavitation and its biological significance.

Investigators have found a number of situations in which biologically significant changes are brought about by ultrasound, and for which neither temperature elevation nor cavitation seems to be the mechanism. In some instances the changes are a result of radiation forces. These forces may cause particles to be displaced from their normal positions and into new configurations which are unfavorable biologically. Also, radiation forces may lead to distortion, and consequent damage, to biological structures. In other situations, the fluid component of a biological suspension is set into small-scale circulatory motion, called acoustic streaming or microstreaming. With these motions are associated hydrodynamic stresses large enough to cause damage to suspended cells and macromolecules. Radiation forces, microstreaming and other related mechanisms for sonic bioeffects are discussed in Section 4.

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* Ultrasound is simply sound whose frequency exceeds a given limit, often taken as 20 kHz. Many of the phenomena produced by sound occur at low frequencies, as well as high. In this report we shall frequently use the terms sound, sonic, etc., in making statements applicable to sound generally.

There are a number of practical motives for studying the biological effects of ultrasound. For example, ultrasound is used in physical therapy (8), and other therapeutic applications show promise (9-11). Studies of bioeffects are needed to make these applications as effective as possible. However, the matter of greatest present concern is undoubtedly one arising from the increasingly widespread use of diagnostic ultrasound. Because of the near-routine clinical use of this technique in obstetrics and gynecology, for example, it appears that a large fraction of the future world population will have been irradiated with ultrasound prenatally. While clinical experience (12-14) does not indicate any danger to mother or child, it is clearly essential to establish the condition for safety on a very reliable basis when so many individuals are involved. Related to this safety question is the possibility that, in the future, Federal standards will be imposed on medical devices, including those for diagnostic ultrasound. (Authority is provided by Public Law 94-295, 94th Congress, S.510, May 28, 1976). In connection with this the question arises, whether the "dose" of ultrasound applied to a patient during examination should be prescribed, in analogy to current practice for prescription drugs. Any satisfactory prescription of this kind should be based on information about the boundaries, if they exist, between values of the ultrasonic parameters which apply when significant bioeffects occur, and those which apply when there are no important bioeffects. Existing information on this subject is reviewed in Section 5.

Our knowledge on bioeffects comes from (i) clinical experience, (ii) laboratory experiments and (iii) applications of established principles. The latter source of information is emphasized in the report. As the mechanisms for the action of ultrasound are identified and understood, it becomes increasingly possible to predict the consequences of applying the physical agent in various ways.

2. SONICALLY GENERATED HEAT

2.1 SOUND ABSORPTION

Of the several major mechanisms by which ultrasound affects biological systems, the best understood is undoubtedly that of temperature elevation caused by sonically generated heat. In this section (Section 2) we first discuss the processes of heat generation by ultrasound. We then treat specific temperature fields which result from the heat generation under several idealized circumstances. In Section 2.4 we obtain results to which we shall refer in discussing available bioeffects data for mammalian systems.

When a plane wave of ultrasound travels through a homogenous medium its intensity I decreases through absorption, the sonic energy being continually converted into heat. If the direction of travel is along x the intensity I at any position x is given by

$$I = I_0 e^{-2\alpha x} \qquad (1)$$

where α is the absorption coefficient (specifically the amplitude absorption coefficient) and I_0 is the intensity at $x = 0$. If x is in centimeters the units of α are "nepers per centimeter" (Np/cm). (The neper is numerically equal to 8.68 decibels (db).) Because of the absorption, $2\alpha I$ units of heat are generated per unit volume per unit time in any region where the intensity is I. If all of this heat remains where it is generated (none of it being transported from one

point to another by heat conduction or other means) the temperature in this region will rise at the rate

$$dT/dt = 2\alpha I/\rho c_m \qquad (2)$$

where dT/dt is the temperature rise in unit time, while ρ is the (mass) density and c_m is the specific heat per unit mass. As an example to illustrate the units we suppose ρ is 1.0 gm/ml and c_m is 1.0 calories (i.e., 4.18 joules) per gram per degree Celsius; these are appropriate as rough values for biological tissue. We let $\alpha = 0.1$ Np/cm, as is approximately correct for liver or brain; if I is 1 W/cm^2 we obtain from Eq. (2) that dT/dt is 0.048 °C/sec or 2.9 °C/min. For short pulses of ultrasound we can expect the temperature to rise at this rate during each pulse. For continuous ultrasound the expression in Eq. (2) will apply only for a short time after a sound field is turned on; soon conduction and other transport processes become significant and Eq. (2) no longer holds. In table 1 are shown a few representative values of α for water and for mammalian tissues.

Table 1. Absorption coefficients at 1 MHz

Medium	α(Np/cm)
Water (degassed)	0.00025
Fat	0.057
Brain	0.1
Liver	0.1
Muscle	0.23
Bone	1
Lung	4

A comprehensive review of the literature on the absorption of ultrasound in tissue and other biological media has been published by Dunn, Edmonds and Fry (15). Shorter reviews and recent contributions are presented in a report by Reid and Sikov (2). A brief discussion of the physical bases for sound absorption appears in Chapter 13 of a textbook by Nyborg (16).

Many experimental and mathematical investigations have been carried out to develop basic understanding of the processes leading to sound absorption. We shall take these up only briefly here. In a Newtonian liquid part of the absorption is associated with viscous shearing action which causes irreversible conversion of mechanical energy into heat. If this were the only mechanism for absorption the coefficient α would be

$$\alpha = 2\omega^2\eta/3\rho c^3 \qquad (3)$$

where ω is the angular frequency (equal to 2π times the frequency f in Hz), η is the coefficient of shear viscosity and c is the velocity of sound. Usually the actual absorption in a liquid is greater than expected from Eq. (3). To account for the total absorption in a formal way the expression $(\eta + (3B'/4))$ is often substituted for η in Eq. (3); here B' is called the coefficient of bulk viscosity. Typically B' is not constant but is a function of freguency. However, for water B' is constant over a wide range of frequency; at 30° C the ratio B'/η is 2.8.

In concentrated biological suspensions, or in tissues, the absorption is much greater than for water. While the basic causes of absorption have not been determined for tissues it is usually assumed that relaxation processes are involved. In a linear relaxation process the medium, disturbed by the sound, continually seeks to return to equilibrium at a rate dependent on the

disturbance. Because the system always tends to be out of equilibrium, a sinusoidally varying pressure of given frequency in the medium is associated with a sinusoidally varying density with the same frequency, but somewhat differing in phase. It is not hard to show that this phase lag between pressure and density leads to an irreversible conversion of mechanical energy into heat, and hence to sound absorption. For some media rather simple relaxation processes take place and have been fully analyzed, but for biological tissues there appear to be multiple processes involved, which have not yet been fully identified.

In some media sound propagation leads to heat generation at inhomogeneities. This is particularly true of lung tissue, whose high absorption coefficient is evidently associated with energy dissipation in the multitudinous small air-filled cavities provided by the alveoli. See Bauld and Schwan (17) and Dunn (18). Related to this is the fact, discussed in Section 3, that isolated gas bubbles can be extremely effective as sites for heat generation in a sound field.

Given that heat is continually being produced in a medium as ultrasound passes through, we now consider temperature fields which result from this. Complications arise from inhomogeneity of the biological tissue and from the non-uniformity of real sound fields. Also in typical medical applications, time variations are introduced by pulsing the ultrasound. Hence in practice the temperature varies in both space and time in a complex way. While the simple expression for dT/dt in Eq. (2) is nevertheless useful for some conditions, it is not adequate in general; this is especially true since the consequences of heat transport have been ignored in arriving at this equation, but must usually be considered. In a solid medium the main transport mechanism is heat conduction, while in a medium containing fluid, heat may be carried along also by convection. The latter process is especially important where there is forced convection, as in a mammalian circulatory system. In the following brief analysis of temperature fields we shall, for simplicity, neglect convection effects. We shall also ignore metabolic and other sources of heat which may exist in a living system, and whose output may be altered in a sound field through bio-regulation.

2.2 POINT SOURCE OF HEAT

Insight on sonically generated temperature fields is gained by taking up special basic situations. First we suppose the heat is generated only in a very small region, near some point P. This will occur, even in a uniform ultrasonic field, if the region near P is characterized by a very high absorption coefficient relative to the surrounding medium. If heat is generated steadily in the vicinity of P, beginning at $t = 0$, we have from a solution of the heat conduction equation, discussed by Carslaw and Jaeger (19), that the temperature T at subsequent time t, at a distance r from P, is given by

$$T - T_0 = (q/4\pi Kr)\ \mathrm{erfc}(R), \tag{4a}$$

where T_0 is the ambient temperature while R is a nondimensional parameter given by

$$R = r/2(\kappa t)^{\frac{1}{2}}. \tag{4b}$$

Here K is the coefficient of thermal conductivity while κ is the thermal diffusivity; the constants K and κ are related by

$$\kappa = K/\rho c_m; \tag{5}$$

for water, K is 0.0060 W/cm °C, and κ is 0.00144 cm^2/sec. The quantity q gives the heat generated in unit time by the heat source at P. The complementary error function appearing in Eq. (4) is defined by the relations

$$\text{erfc}\,(R) = 1 - \text{erf}(R) = \frac{2}{\sqrt{\pi}} \int_R^\infty e^{-u^2}\, du \,. \qquad (6)$$

The function erfc(R) is unity at small values of R, and falls monotonically to zero as R increases. At R = 0.2, 0.5, 1.0 and 2.0 the function erfc (R) has the values 0.78, 0.48, 0.157, and 0.005, respectively.

In figure 1 are plots on log-log scales of $(T - T_0)$ versus r for several values of t; for these, K and κ are given the values for water. For convenience we set q = 75.4 μW, so that $q/4\pi K$ is numerically equal to 0.001 cm °C. The quantity $(T - T_0)$ plotted in Fig. 1 then gives the temperature elevation in degrees Celsius at various distances (in cm) from a point heat source generating 75.4 μW. At small values of r, the temperature elevation $(T - T_0)$ falls off inversely with r, following the dashed ("1/r") curve (a straight line on this log-log plot) shown on figure 1. At larger distances r the fall-off is more rapid. To see the trend, consider points corresponding to characteristic values of the nondimensional parameter R. When R = 1 the actual distance r is $2\sqrt{\kappa t}$, equal to $0.076\sqrt{t}$ centimeters in water; at this distance $(T - T_0)$ is about 16 percent of the value given by the "1/r" curve (for which R = 0). This condition is marked on each of the solid curves by its intersection with the dashed curve R = 1. When R = 2, corresponding to $r = 0.152\sqrt{t}$ centimeters, the temperature elevation is only 0.5 percent of the 1/r value; see intersections with the dashed curve R = 2.

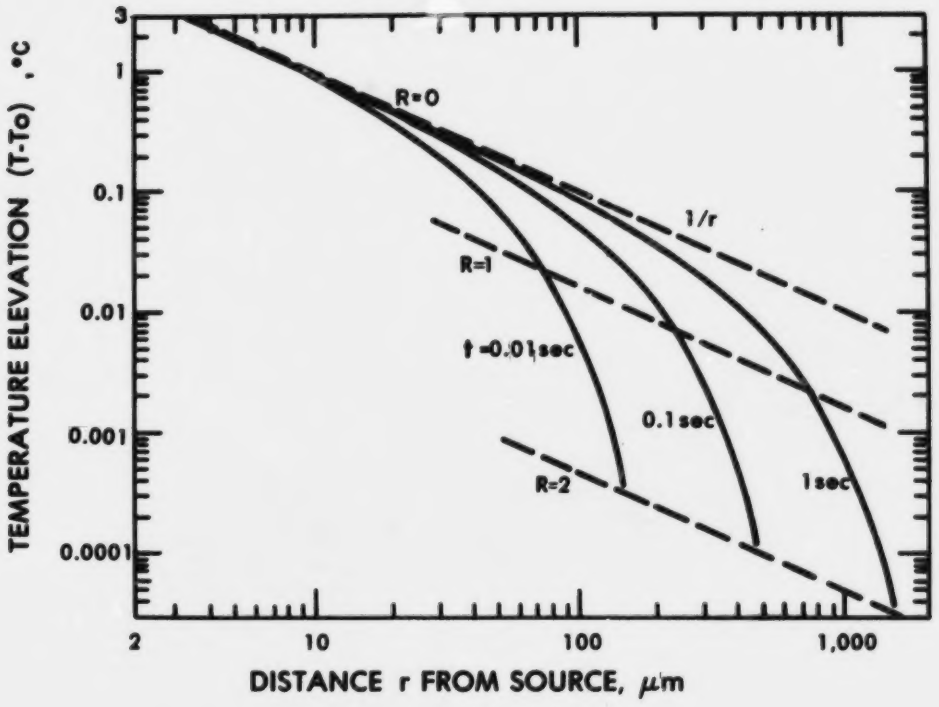

Figure 1. Temperature elevation ($T - T_0$) in degrees Celsius near a point source of heat which generates the heat at a constant rate of 75.4 μW, beginning at time $t = 0$. As time goes on, each characteristic point (a point at which ($T - T_0$) is less by a fixed ratio than the "1/r" value) moves outward, its distance r from the source being proportional to $\sqrt{t}$. Dashed lines show the conditions R = 0, 1, 2, respectively, where R is a "nondimensional distance" defined in the text.

In figure 2 are plots of ($T - T_0$) versus t for several values of r, again based on the small-source solution, Eq. (4). At any given distance r from the origin the temperature elevation rises rapidly at first (large slope on the log-log plot), then more slowly, leveling off to the value $q/4\pi Kr$. The time required for ($T - T_0$) to reach half its final value is approximately equal to r^2/κ; in water this time (in seconds) is about 694 r^2 when r is in centimeters. The ordinate gives ($T - T_0$) in degrees Celsius when $q/4\pi K$ has the same value as in figure 1.

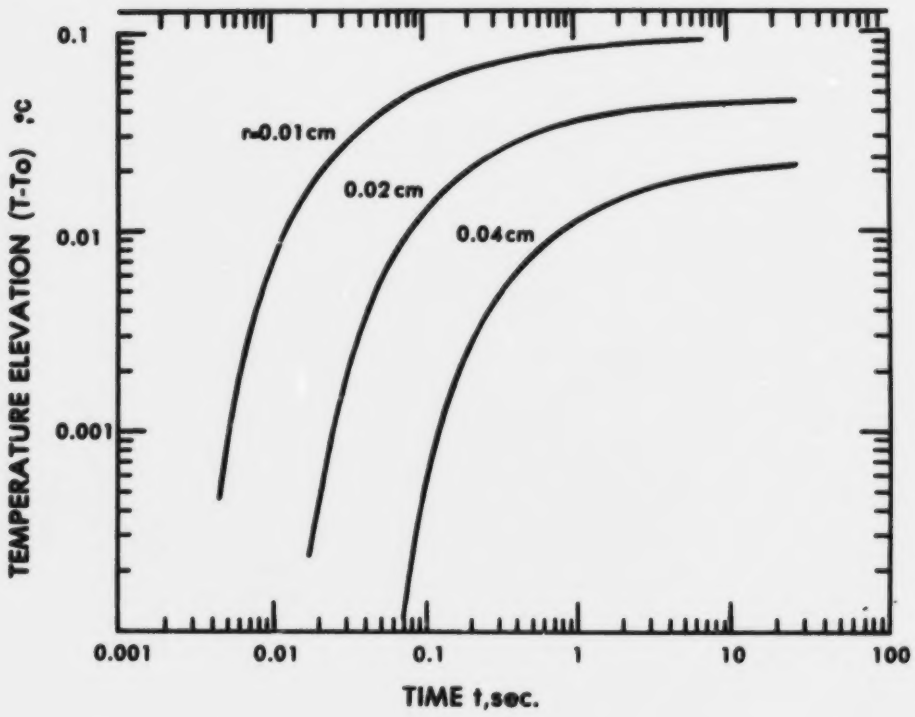

Figure 2. Temperature elevation near point source of heat as in Fig. 1, but here the plots are of $(T - T_0)$ versus time.

2.3 SPHERICAL SHELL AS HEAT SOURCE

We now take up another basic expression which satisfies the heat conduction equation; here we suppose a thin spherical shell acts as a steady heat source, beginning at $t = 0$. This result gives insight on possible situations where ultrasound impinges on a body whose surface is highly absorbing compared to its interior, as well as to the surrounding medium. For a shell of mean radius a we have (19)

$$T - T_0 = (q/8\pi Kar)(X - Y), \tag{7a}$$

where q is the rate of heat generation by the entire shell, r measures distance from the center of the shell and

$$X = 2(\kappa t/\pi)^{\frac{1}{2}} \{e^{-(R-A)^2} - e^{-(R+A)^2}\};$$

$$Y = -|r - a| \operatorname{erfc} |R - A| + (r + a) \operatorname{erfc} (R + A); \tag{7b}$$

$$R = r/2(\kappa t)^{\frac{1}{2}}; \quad A = a/2(\kappa t)^{\frac{1}{2}};$$

other symbols are as defined previously. In figure 3 is a plot of $(T - T_0)$ versus r for $a = 1$ cm and at a number of different times t. As before κ is

chosen for water but now q is set equal to $8\pi K$ in the units chosen; hence figure 3 applies, with $(T - T_0)$ in degrees Celsius if the shell generates 151 milliwatts of heat. We see that at t = 10 sec the temperature has risen noticeably in the vicinity of the shell at r = a, but no temperature rise is observable at more than a few millimeters inward or outward from r = a. At 100 seconds the temperature at the center r = 0 has risen noticeably. The temperature at r = 0 increases rapidly as the time increases to 316 seconds; also, by that time the interior temperature T_i has become nearly uniform inside the shell. With further increase of time, T_i becomes increasingly uniform while the exterior temperature tends to fall off with distance according to 1/r. At very large values of time T_i approaches the limiting value of 2° C.

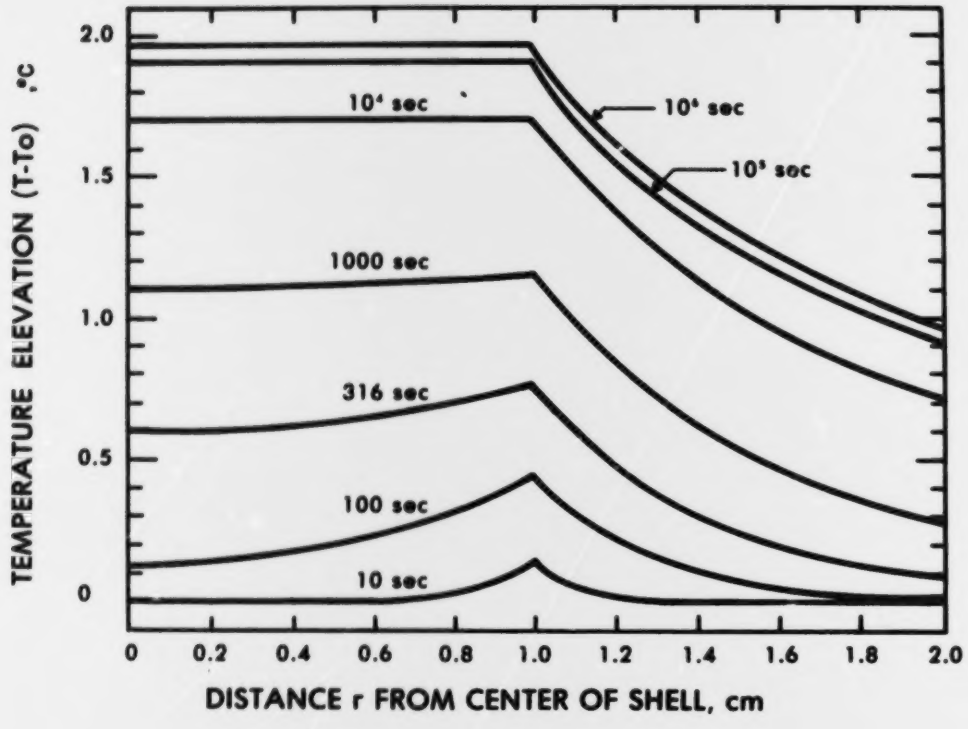

Figure 3. Temperature elevation within and without a heated spherical shell of radius 1 cm. $(T - T_0)$ is in degrees Celsius if the rate of heat production is 151 mW. On each curve is shown the time elapsed since the heating began.

At the center r = 0 the expression in Eq. (7) simplifies considerably and yields

$$(T - T_0)_{r=0} = (q/4\pi Ka)\ \mathrm{erfc}(A), \qquad (8)$$

where A is defined in Eq. (7b). The similarity of Eq. (8) to Eq. (4a) (for a "point" source) is interesting; plots of $(T - T_0)$ versus t from Eq. (8) would obviously be like the plots in figure 2, except that a would replace r as parameter, and A would then replace R.

2.4 SPHERICAL HEAT SOURCE

The expression for the spherical shell in Eq. (8) can be used to find the temperature at the center of any spherically symmetrical heat source, which has been generating heat steadily since t = 0. For the special case of a sphere of radius a an integration based on Eq. (8) yields

$$(T - T_o)_{r=o} = 4\rho c_m q_v t \int_o^A u \operatorname{erfc} u \, du \; ; \qquad (9)$$

here q_v (assumed constant throughout the sphere) gives the rate at which heat is generated per unit volume, and other symbols have been defined previously. If the sphere is uniformly irradiated by a plane wave of intensity I, and if the absorption coefficient in the sphere is α (with $\alpha a << 1$) the quantity q_v will be approximately

$$q_v = 2\alpha I. \qquad (10)$$

At small values of t, the parameter A is very large, and the integral on the right hand side of Eq. (9) approaches the value (1/4). Using Eq. (10) for q_v we then obtain

$$(T - T_o)_{r=0} = 2\alpha I t/\rho c_m. \quad \text{(Small t)} \qquad (11)$$

This result, showing the temperature elevation $(T - T_o)$ increasing linearly with time for small t, is as anticipated in Eq. (2). At large values of the time, A is very small and the integral on the right of Eq. (9) approaches $A^2/2$. Then $(T - T_o)$ is independent of time and given specifically by

$$(T - T_o)_{r=0} = \alpha I a^2/K. \quad \text{(Large t)} \qquad (12)$$

This result can be obtained independently in a simple way, by considering time-independent conduction, as it occurs after sufficient time has elapsed (20). In the steady state field that then applies the temperature elevation $(T - T_o)$ is maximum at r = 0, and given by Eq. (12); at the edge of the sphere r = a the quantity $(T - T_o)$ is reduced to two thirds of this maximum value.

In figure 4 are shown plots, on log-log scales, of $(T - T_o)$ at r = 0 as a function of the time t, based on Eq. (9). The three plots are for different spherical radii, given by a = 1 cm, 2 cm and 4 cm, respectively. To evaluate constants in Eq. (12) it was assumed that the sphere is composed of biological tissue similar to liver or brain, with α=0.1 Np/cm at 1 MHz; the intensity was taken to be 0.1 W/cm^2 while K was chosen as for water. Examining any of the plots we see that, as anticipated, $(T - T_o)$ increases linearly at first, then rises at a decreasing rate, leveling off to the value given in Eq. (12). For a = 1 cm the final temperature rise is 1.67° C. In a time t = 700 a^2 (i.e., t = a^2/κ), where a is in centimeters and t is in seconds, the temperature elevation has risen to within about 20 percent of its final value. Both the limiting temperature rise and the characteristic time are proportion to a^2. In Section 5 we shall see that this theory for temperature elevation in a heated sphere is useful in fitting data on intensity and time for bioeffects in tissues.

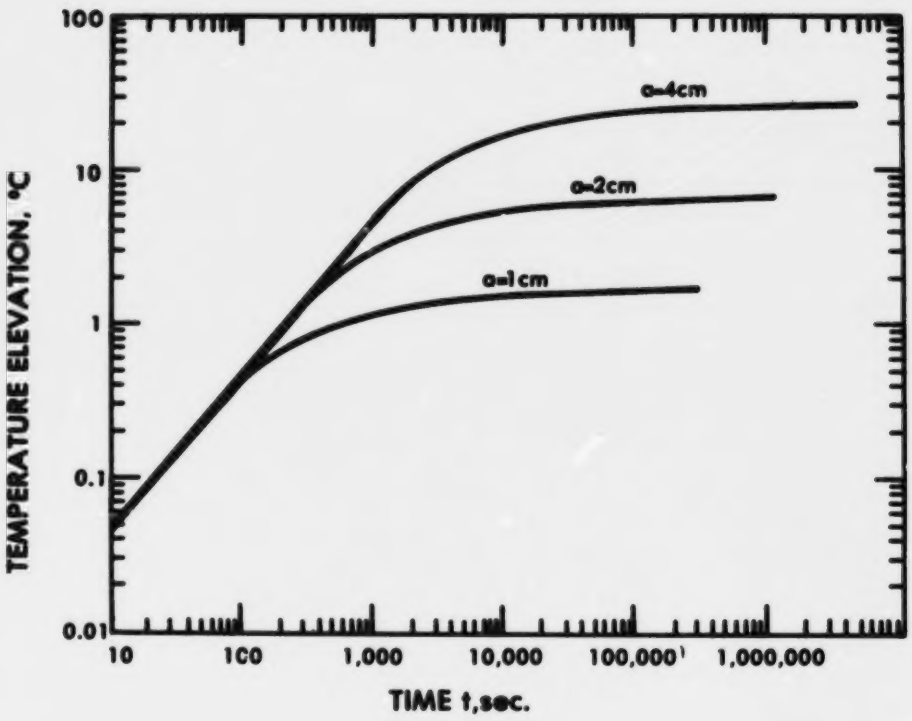

Figure 4. Temperature elevation at the center of a uniformly heated sphere of radius a. The sphere is assumed to be an absorber with $\alpha = 0.1$ Np/cm^2 on which is incident an ultrasonic wave of intensity 100 mW/cm^2.

3. CAVITATION

When biological cells or macromolecules are in aqueous suspensions and subjected to ultrasound they may be altered structurally, or functionally, or both. This may occur even when significant temperature elevation is prevented, either (i) by cooling the suspension or (ii) by pulsing the ultrasound so that heat is conducted away from the test region between pulses. Under such conditions the cellular or molecular alterations are nearly always associated with "cavitation"; by this we mean an activity of highly compressible bodies (or "bubbles" or "cavities") composed of gas and/or vapor. This activity may be gentle or violent; it may be relatively simple in nature, but is often very complex. For understanding biological effects which occur at low intensity levels our interest is especially with the subtler manifestation of cavitation. The treatment here is rather brief. An extensive review of cavitation phenomena has been given by Flynn (21); a review specifically oriented toward biomedical applications is cited in Reference 22.

3.1 BREATHING OSCILLATIONS; RESONANCE

In its simplest form the active element is a sphere of gas. In a sound field the bubble executes breathing pulsation in response to the applied pressure oscillations; its radius contracts to $R_0 - \xi_0$ and expands to $R_0 + \xi_0$

once during each sonic cycle, where ξ_0 is the radial displacement amplitude, and R_0 is the mean bubble radius. At low frequencies the applied pressure is in phase with the pressure in the bubble. Hence the radius (and thus also the volume) has its minimum value when the applied pressure is at its maximum and, conversely, is greatest when the applied pressure is least. Changes in state of the gas take place isothermally under extreme conditions where the bubble is very small and the frequency very low, and adiabatically under the opposite extreme conditions. In general the conditions are intermediate, and the process is neither completely adiabatic nor isothermal.

At very high frequencies the applied pressure and the pressure in the bubble differ in phase by 180°; hence the volume increases when the applied pressure increases, etc. This change in phase reflects the existence of a resonance characteristic for the breathing pulsation of a gas bubble. The dynamical situation presented by a gas bubble in a liquid is analogous to that of the mass-plus-spring model referred to in elementary physics. The gaseous sphere acts like a spring while a portion of liquid surrounding the sphere behaves like a mass. In water, for an air bubble that is not too small, the resonance frequency f_0 is often given adequately by

$$f_0 = 3260/R_0; \qquad (13a)$$

here f_0 is in kHz when R_0 is in μm; adiabatic conditions are assumed. Thus a bubble 100 μm in radius is resonant at 32.6 kHz. Equation (13a) is appealingly simple but, unfortunately, is not accurate for gaseous spheres of the small size corresponding to resonance in the megahertz range. An equation which applies more generally (21,22) is

$$\rho_o \omega_o^2 R_o^2 = 3\gamma\left(P_o + \frac{2\sigma}{R_o}\right) - \frac{2\sigma}{R_o} \; ; \qquad (13b)$$

here ρ_0 is the density of water; $\omega_0 = 2\pi f_0$ is the angular frequency at resonance; γ is the "effective ratio of specific heats" or the "polytropic index" for the gas; P_0 is the hydrostatic pressure and σ is the surface energy (i.e., "surface tension") of an air-water interface. Equation (13b) reduces to (13a) when (i) σ/R_0 is much less than P_0 and (ii) γ is approximately 1.4. The latter assumption ($\gamma = 1.4$) applies for air when the volume changes adiabatically, as is approximately true for resonant air bubbles in water at frequencies up to a few hundred kilohertz. In the megahertz range the adiabatic condition does not hold; the effective value of γ is not accurately known, but for air it must lie between an upper limit of 1.4 and a lower limit of unity. The latter limit applies to very small bubbles which oscillate isothermally. A range of possibilities is indicated in table 2 where resonance frequencies are tabulated for a few values of the radius R_0; Eq. (13b) is used with two values of the surface tension σ and three values of the index γ.

In this table the first line ($\gamma = 1.4$, $\sigma = 0$) applies with sufficient accuracy for air bubbles in the lower frequency range; for this choice of parameters Eq. (13b) reduced to Eq. (13a). For each of the lower three lines σ has the value 73 dyn/cm, as is appropriate for an air-water interface at room temperature. For these three lines the index γ is assigned, in turn, its maximum value (1.4), its minimum value (1.0), and an intermediate value (1.2). For $\gamma = 1.4$ comparison can be made between the resonance frequencies for $\sigma = 0$ and $\sigma = 73$ dyn/cm; we see that surface tension is especially important at the smaller values of R_0, but is significant even for R_0 as large as 4 μm. Decrease of γ leads to a lowering of the resonance frequency.

It has been found recently by D.L. Miller (21a) that at a frequency of 1 MHz a free air bubble is resonant at about $R_0 = 3.5$ μm. He pointed out that this is consistent with available theory if γ is chosen as 1.2. Miller's result is approximately in accordance with table 2, for there we see that $R_0 = 3.5$ μm and $\gamma = 1.2$ correspond to a resonance frequency of 0.98 MHz.

Table 2. Calculated resonance frequencies in MHz for small air bubbles. σ in dyn/cm. R_0 in μm.

	$R_0 = 1.0$	1.2	1.6	2.0	3.0	3.5	4.0
$\gamma = 1.4$ $\sigma = 0$	3.26	2.72	2.04	1.63	1.09	0.93	0.82
$\gamma = 1.4$ $\sigma = 73$	4.74	3.78	2.66	2.04	1.27	1.07	0.92
$\gamma = 1.2$ $\sigma = 73$	4.33	3.45	2.43	1.87	1.17	0.98	0.85
$\gamma = 1.0$ $\sigma = 73$	3.87	3.09	2.19	1.68	1.06	0.89	0.77

We shall now be more specific in describing the response of a gas bubble to a sound field. Suppose that at the site of the bubble, but in its absence, there exists an ultrasonic field in which the pressure varies sinusoidally in time with frequency f and amplitude p_0; this ultrasonic field is the driving field. Then for a bubble of resonance frequency f_0, the phase difference between its volume and the pressure in the driving field is 180° if $f \ll f_0$ and is 0° if $f \gg f_0$. The phase difference makes its transition from 180° to 0° in the vicinity of the resonance condition and is just 90° when $f = f_0$.

We consider now the amplitude ξ_0 of the radial displacement. Here we find it convenient to refer to a nondimensional or relative frequency, namely, the ratio

$$\Omega = f/f_0. \tag{14}$$

From theory described elsewhere (22) it is found (neglecting effects of surface tension for this purpose) that

$$\xi_0 = \xi_{00}\, \chi(\Omega), \tag{15a}$$

where

$$\begin{cases} \xi_{00} = p_0 R_0 / 3\gamma P_0 \\ \chi = \left((1 - \Omega^2)^2 + \Omega^2 \delta^2\right)^{-1/2} \end{cases} \tag{15b}$$

In these equations δ is the damping constant, a measure of energy dissipation equal to the reciprocal of the "quality factor" Q sometimes referred to in discussing oscillations. Other symbols have the same meanings as before. It is clear from Eqs. (15) that ξ_{00} is the value of ξ_0 at low frequencies ($\Omega \ll 1$); also χ is the ratio ξ_0/ξ_{00} which we shall call the relative amplitude. In figure 5 are seen plots of the relative amplitude ξ_0/ξ_{00} against the relative frequency $\Omega = f/f_0$. At low frequencies ($\Omega \ll 1$; i.e., $f \ll f_0$) the relative amplitude is unity, as is required from the definition of ξ_{00}. From Eqs. (15) we see that when $\Omega = 1$, corresponding to $f = f_0$, the relative amplitude χ has the value $1/\delta$; this is (approximately) the value of χ at the maxima exhibited on the plots of figure 5. For a resonant bubble at 1 MHz the damping constant δ is estimated (22) to be 0.14. Hence for this condition the maximum value of χ is about 7, a value intermediate between those for the plots shown in figure 5. At high frequencies ($\Omega \gg 1$, $f \gg f_0$) the relative amplitude χ becomes much less than unity; i.e., ξ_0 becomes much less than the low-frequency value ξ_{00}.

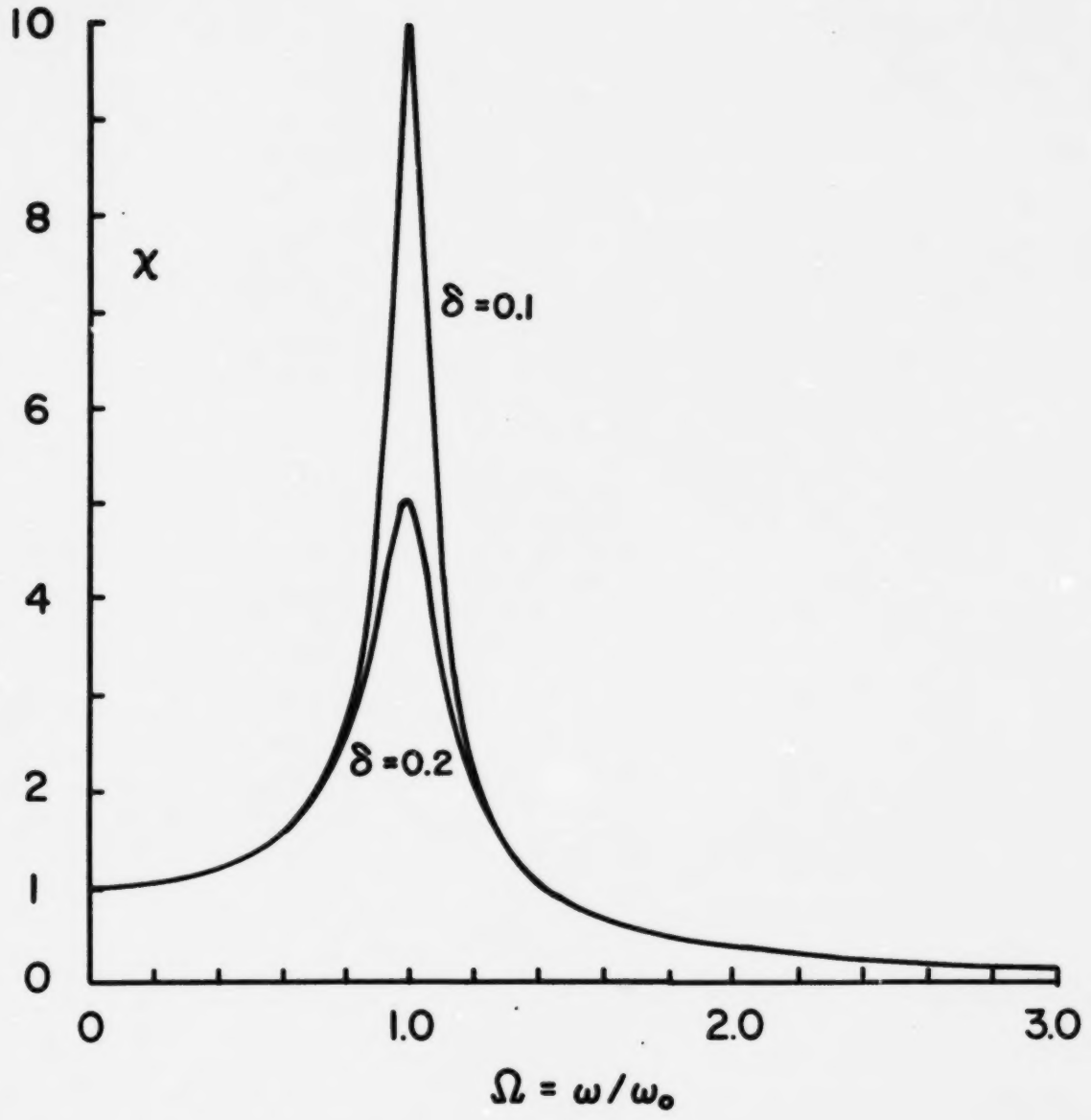

Figure 5. Relative amplitude χ for a pulsating bubble versus relative frequency Ω, based on Eq. (15), for two values of the damping constant δ.

The theory given in this section (Section 3.1) is for the idealized situation of a spherical gas bubble in an "infinite" liquid medium, i.e., well away from boundaries. For a bubble near a vessel wall, or trapped in a crevice, the resonance frequency may be significantly altered. See Howkins (23).

3.2 EXTRACTION OF ENERGY; SOUND ABSORPTION

As a consequence of its vibration a bubble extracts energy from the sound field which drives it; some of this energy is converted irreversibly into heat, and another part is re-radiated from the bubble as an outgoing spherical wave. Specifically, calculations show that in water a resonant bubble at 1 MHz in a driving field of intensity 100 mW/cm^2 removes about 60 μW from the field. Of this about 90% is converted into heat, and the remaining 10% is re-radiated, i.e. scattered. When the intensity I is low enough the power removed is proportional to I; at higher intensities the relationship is nonlinear.

If the biological tissue in a given region contains a distribution of such bubbles it can absorb sound readily, and thus act as an effective heat source. An interesting hypothesis once suggested by Dunn and Fry (though later modified) (18) for the very high absorption coefficient of lung tissue is that it is caused by gas bubbles. To illustrate possibilities for any model of this kind we have calculated that if 1,000 bubbles were present per cubic centimeter, all resonant at a frequency of 1 MHz, the absorption coefficient would be about 0.6 Np/cm; these bubbles would occupy only a tiny fraction (less than 10^{-6}) of the total tissue volume. We saw in Section 2 that when sound passes through a medium the rate of heat generation is proportional to the absorption coefficient. Hence we expect heating to occur preferentially in any tissue containing an appreciable number of bubbles resonant to the sound.

3.3 RECTIFIED DIFFUSION

An important aspect of the behavior of small gaseous bodies or bubbles in liquid is that, under suitable circumstances, they will grow in a sound field. This growth is associated with diffusion of gas through the liquid surrounding the bubble. Oscillation of a spherical bubble causes gas to diffuse radially inward and outward during each cycle, but in an asymmetrical manner so that net inward flow results. Growth of the bubble occurs when this sonically produced inward flow exceeds the outward diffusion flow; the latter is assumed to occur as it would if the sound were absent. Such growth is attributed to rectified diffusion and is important in the dynamics of sonic cavitation.

For maintenance of cavitation action in a liquid at ultrasonic frequencies and at low or moderate levels it appears to be necessary for the liquid to contain rather small gas bubbles, lying in a preferred size range. Free bubbles of optimum size for megahertz frequencies are not likely to persist in water in the absence of sound, since they tend to shrink and disappear by diffusion unless they are stabilized in some way. (See Section 3.9). Rectified diffusion aids the cavitation process by preventing desired bubbles from shrinking away. If the only gaseous bodies present before sonication are smaller than desired (as is often true of tiny bubbles trapped in cracks, etc.) these may be grown to optimum size by rectified diffusion.

In order for bubbles of desired size to be produced or maintained by rectified diffusion the sound level must exceed some threshold value (21, 22). This threshold is evidently also the minimum level at which cavitation activity will take place in a liquid, if rectified diffusion is the only mechanism for providing the required bubbles.

3.4 RADIATION FORCE ON A GAS BUBBLE

In a typical sonic cavitation field there is considerable motion of the active bubbles; these motions are important in affecting the spatial and

temporal distributions of cavitation activity. Some aspects of the motions can be accounted for in terms of acoustical theory for radiation forces on spherical gas bubbles. The subject is reviewed in Reference 22. We shall not discuss the theory here but instead refer to some of the more interesting results:

(i) In a standing wave field, radiation force causes a bubble smaller than resonant size to move in the direction of increasing pressure amplitude, and a bubble larger than resonant size to move in the opposite direction. Thus small bubbles ($\Omega < 1$) tend to collect at maxima of the pressure amplitude, and large ones ($\Omega > 1$) at the corresponding minima. There is no force on a resonant bubble in this kind of a field.

(ii) Two bubbles attract each other if they are equal in size or, whether equal or not, if both are smaller than resonant size. If one bubble is larger and the other smaller than resonant size, they repel at large separations but may attract at small distances.

(iii) In a progressive wave, radiation force propels the bubble along the direction of propagation; the force is maximum if the bubble is resonant.

It was noted in connection with (i) that bubbles smaller than resonance size move to presssure maxima in a standing wave. There they tend to vibrate vigorously in response to the relatively large pressure amplitude. They may then grow by coalescence with other small bubbles (by virtue of (ii)), or by rectified diffusion. When they grow past resonant size they leave the pressure maximum (by virtue of (i)); often they move to a pressure minimum where they are quiescent.

The characteristics described above explain some observations of Goldman and Lepeschkin (24). Positioning plant filaments along a standing wave they found that damage to the cells occurred principally near planes one-half wavelength apart, corresponding to pressure maxima. This result is resonable since, as explained in the previous paragraph, maximum cavitation activity is to be expected at the pressure maxima.

3.5 MICROSTREAMING NEAR A PULSATING BUBBLE

When a gaseous body pulsates its motion is not usually spherically symmetric, either because of distortion by an adjoining solid boundary, or because of surface waves set up by the sound field. Asymmetric or nonuniform oscillation of the air-liquid interface at the surface of an airpocket or bubble causes steady eddying motion to be generated in the immediately adjoining liquid; since this motion is typically of small scale it is appropriately called microstreaming. When the air pocket is at the surface of a solid boundary (such as a container wall or the exterior of a suspended rigid particle) the microstreaming gives rise to high velocity gradients at the boundary. If biopolymer molecules or small biological cells are suspended in the liquid they may be swept into a region of high velocity gradient. Here they are subjected to shearing action and damage may occur, such as fragmentation of macromolecules and membranes. Effects of shearing action promoted by sound are taken up in Section 4. Significant bioeffects are expected in suspensions near resonant bubbles, even at low intensity levels.

3.6 RADIATION FORCE ON A PARTICLE NEAR A PULSATING BUBBLE

When a gaseous body vibrates in a breathing mode it tends to collect nearby suspended particles, provided these are more dense than the solvent liquid.

According to theory for acoustic radiation pressure (22) the speed v with which the particle approaches a spherical bubble is given by

$$v = \frac{3\rho_0 s\omega^2 \xi_0^2 R_0^4}{(2\rho + \rho_0) r^3} \quad ; \qquad (16)$$

here ρ_0 and ρ give the density for the medium and the particle, respectively; ω is the angular frequency; R_0 is the mean radius of the bubble; ξ_0 is the vibration amplitude of the bubble as defined in Section 3.1; r is the distance of the particle from the center of the bubble; and s is the sedimentation coefficient (25) given for a spherical particle of radius a by

$$s = \frac{2a^2}{9\eta} (\rho_0 - \rho), \qquad (17)$$

where η is the shear viscosity coefficient for the liquid. As an illustration, from the theory in Section 3.1: At a frequency of 1 MHz in water a driving pressure amplitude of less than 0.05 atm will set a resonant bubble ($R_0 \simeq 3.2$ μm) into oscillation with an amplitude ξ_0 equal to 0.1 R_0. For a representative biomolecule (such as a blood protein) we choose s as 5×10^{-13} sec. Setting $r = R_0$ to obtain the largest value of v we calculate about 0.5 μm/sec. Hence such a vibrating bubble in a suspension (e.g., blood plasma) containing proteins would probably become coated with these molecules in a few seconds. Larger particles would be drawn to the bubble in a shorter time. (However Eq. (16) is strictly valid only when the particle radius a is much less than the bubble radius R_0).

3.7 SURFACE WAVES AND MICROBUBBLE GENERATION

Some aspects of cavitation are dependent on nonuniformity in the motion of the bubble surface. We have already noted (Section 3.5) that microstreaming is produced near a bubble vibrating nonuniformly. For example, Gould (26) has investigated changes in transport of heat and matter produced by microstreaming near a bubble on which surface waves are established. These waves may be small perturbations at the gas-liquid interface, with a simple sinusoidal profile, or they may be of high amplitude, assuming highly contorted and unstable configurations. (The subject of surface waves is reviewed in Reference 22.) At low amplitudes the displacement pattern on the surface of a gaseous sphere may be represented in terms of one or more spherical harmonics. If ξ_{mn} is the instantaneous radial displacement of the surface in a mode corresponding to the (m,n) harmonic we can write, for example,

$$\xi_{mn} = A_{mn} \cos m\phi \; P_n^m(\mu) \cos \omega_{mn} t; \qquad (18)$$

here m and n are integers; μ is equal to $\cos\theta$; θ and ϕ are the polar and azimuthal angles, respectively; $P_n^m(\mu)$ is a Legendre function of μ; A_{mn} is a constant; and ω_{mn} is the angular frequency of the surface wave. When symmetry exists about an axis we have m = 0 and set ω_{mn} equal to $\omega_{on} = \omega_n$ and A_{mn} equal to $A_{on} = A_n$. Suppose the sound field driving the bubble is of angular frequency ω and pressure amplitude p_0. Then under some circumstances A_n is proportional to p_0 and ω_n is equal to ω; for given p_0 the amplitude A_n has its largest value when ω is equal to one of the characteristic angular frequencies given (27) by

$$\omega_{nc}^2 = (n-1)(n+1)(n+2)\, \sigma/\rho_0 R_0^3, \qquad (19)$$

where σ is the surface tension at the gas-liquid interface. For an air bubble of given radius in water the lowest characteristic frequency for a distortion mode is that for n = 2. If $\omega_{2c}/2\pi$ is to be 1 MHz or less, calculations from Eq.

(19) show that R_o must be 2.8 μm or greater. If $\omega_{2c}/2\pi$ is to be 0.5 MHz, then R_o must be, at least, 4.5 μm.

Under other circumstances the amplitude A_n is essentially zero until p_o exceeds a threshold value p_{ot}, then rises rather abruptly; the surface wave is then said to be parametrically excited. For a surface mode (say, with indices 0, n) established parametrically the threshold p_{ot} is often relatively small when the characteristic frequency ω_{nc} (Eq. (19)) is approximately equal to half the driving frequency ω; it is then typically true that the angular frequency with which the surface oscillates is exactly equal to the subharmonic frequency $(\omega/2)$. If the driving frequency is 1 MHz, so that the "$\omega/2$" subharmonic frequency is 0.5 MHz, we have from the previous paragraph that the radius R_o must be 4.5 μm or greater if a mode for n equal to or greater than 2 is to respond with its characteristic frequency. For any smaller bubble, parametric excitation of a surface mode would be more difficult.

Responses at other subharmonic frequencies (such as $\omega/3$) and at multiples of the subharmonic (e.g., $3\omega/2$) are also possible, but typically require higher driving amplitudes.

When the surface waves are of high amplitude their shape is no longer sinusoidal. In experiments with large air bubbles in gels at low frequencies Storm (28) has shown that evaginations are formed during part of each sonic cycle. See figure 6. Thus air is projected outward into the surrounding medium in relatively thin fingers or filaments. When one of the air-filled filaments extends outward during part of a cycle, an enlarged portion at the end farthest from the bubble may separate. This separated gas volume rounds up and persists thereafter as a new bubble of small size, which is appropriately called a microbubble. As the process continues many microbubbles are generated, and a cloud of them is often formed around the parent bubble. In a liquid this cloud is a very active region in which microbubbles move rapidly because of interbubble radiation forces. Some of them combine with each other to form larger bodies; these, in turn, may combine further, may generate more microbubbles, or may combine with the parent bubble. Some of the smaller microbubbles dissolve in the liquid. A dynamic equilibrium is established in which the amount of gas in the parent bubble and that in the cloud are each maintained at a constant average value. Thus air loss from the parent by microbubble production is restored partly by rectified diffusion and partly by recombination; air gain to the microbubble cloud is balanced by losses through recombination with the parent, and by dissolution.

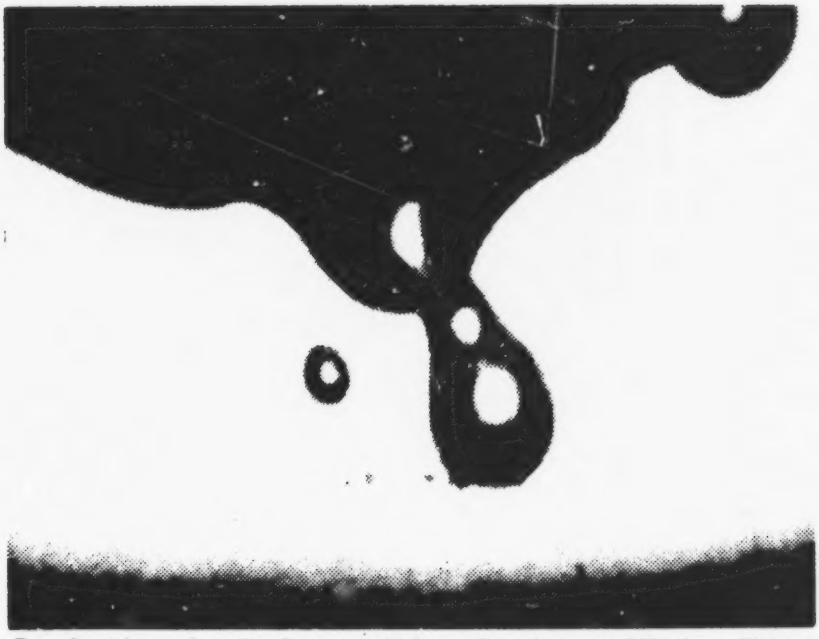

Figure 6. Evagination of air from bubble pulsating at 325 Hz. Photograph is frame of motion picture taken at 4200 frames per second from Storm (Ref. 28).

In some situations microbubble production does not occur at a constant rate but is part of a cyclic process. Neppiras and Fill (29) describe conditions under which small bubbles combine to cause growth of a dominant bubble to resonant size, after which the latter bubble "explodes" into microbubbles and the process begins again.

Microbubbles appear to be important in the "Willard events" which occur in liquids irradiated with focused ultrasound. The significance of these events has been investigated extensively by Coakley and co-workers; their nature is made especially clear in a recent paper by Neppiras and Coakley (30).

3.8 NONLINEARITY; TRANSIENT CAVITATION

Here, as previously in Section 3, we consider a gaseous sphere responding to a driving sound field in which the pressure varies sinusoidally in time with angular frequency ω and pressure amplitude p_0. We suppose that the sphere, i.e., bubble, expands and contracts with spherical symmetry; thus there are no surface waves such as were taken up in Section 3.7. However, we now consider relatively large values of p_0 and, in this way, encounter aspects of bubble dynamics which did not appear in Sections 3.1 - 3.6. In those earlier sections the radial displacement of any point on the bubble surface was assumed to vary sinusoidally in time, with angular frequency ω and with amplitude proportional

to the driving pressure amplitude p_0. Thus a linear relationship was assumed between the displacement and the driving pressure.

In reality, however, even moderate driving pressure amplitudes lead to manifestations of nonlinearity. For example, the pressure spectrum revealed by a small hydrophone in a liquid containing gas bubbles may exhibit superharmonics, subharmonics and/or noise at pressure amplitudes significantly less than 1 atm. Correspondingly the nonlinear theory which applies to a spherical gas bubble in a sound field shows that under the same conditions the radius is no longer a simple sinusoidal function of time. When the driving pressure amplitud p_0 is increased until it is approximately equal to atmospheric pressure or greater, the bubble response may change in character and exhibit a "collapse." Here the radius R increases at a moderate rate during part of a sound cycle, as the bubble expands; then it suddenly contracts, so rapidly that dR/dt approaches the velocity of sound. The temperature in the gas may reach thousands of degrees Celsius and the pressure there may reach several thousand atmospheres. When the "collapse" phenomena occurs, Flynn (21) refers to the activity as "transient" cavitation. It is assumed that after a collapse event the bubble disintegrates, possibly by fragmenting into microbubbles. By contrast, an activity in which gas bubbles are maintained in a state of oscillation for many cycles is called "stable" cavitation.

Either transient or stable cavitation is a potential agent of change in biological solutions or suspensions. In stable cavitation the action may take place through heating, through radiation forces, or through acoustic microstreaming. In transient cavitation the action is relatively violent; bioeffects may be produced by mechanical shocks produced during supersonic contractions, or they may be produced chemically. Chemical changes are often mediated by the free radicals H and OH; the latter probably result from dissociation of H_2O vapor during the contracted, high-temperature, part of the bubble cycle. (See Section 3.10).

3.9 CAVITATION NUCLEI

If stable cavitation is to proceed vigorously, it is clear that gas bubbles must be present of suitable size. For transient cavitation the situation is more complicated, but in most experience this activity also requires the presence of cavities of an appropriate size, filled with gas or vapor.

In freshly aerated water there are many bubbles. The largest of these rise quickly through buoyancy, and leave the liquid at its upper surface. At megahertz frequencies the bubbles of special interest are small, of the order of microns in diameter. Such small bubbles are not usually lost through buoyancy (since they rise very slowly) but nevertheless are not likely to persist long. They are short-lived as a result of diffusion which occurs because of an excess pressure developed in the bubble. This pressure difference Δp is associated with surface tension and for a spherical air-liquid interface is given by $2\sigma/R$ where σ is the surface tension at the interface and R is the radius of curvature of the interface. If R is 3 μm and σ is 80 dyn/cm we find Δp = 0.53 atm. Outward diffusion, driven by the pressure difference Δp (if the outer liquid is just saturated with the gas), causes a micron-sized bubble to dissolve (i.e., to shrink and disappear) rather quickly. The time for complete dissolution varies from a few seconds (for diameters appreciably greater than 1 μm) to a few milliseconds (for diameters less than 1 μm).

Since cavitation activity is readily established in fluids at megahertz frequencies under some conditions, it follows that gaseous or vaporous bodies of appropriate size exist in the fluid under those conditions. One way of

providing such small bodies is by microbubble generation, i.e., by fragmentation of larger gaseous bodies, as described in Section 3.7. These microbubbles will quickly dissolve, as mentioned earlier, if they are free spherical bodies and if the sonic amplitude is low. If the sonic amplitude is sufficiently high, they will be maintained by rectified diffusion (Section 3.3).

Harvey (31) pointed out that "cavitation nuclei," i.e., small bodies of gas, can be maintained indefinitely in the absence of sound if the gas is trapped in cracks or corners. Suitable crevices usually exist in container walls and also on surfaces of small solid particles which exist in almost any liquid unless it has been prepared with special care. In such a crevice the curvature of the gas-liquid interface may be very small, or zero, even though the gas volume is small. When the curvature is small, the radius of curvature is large and the term $2\sigma/R$, representing excess pressure in the gas phase, is small. Hence little diffusion occurs and the gaseous body has negligible tendency to dissolve. In the presence of a sound field of sufficiently high amplitude, gas will be pumped into the body by rectified diffusion, and it may grow to a bubble of optimum size for cavitation. The trapped gas is thus a "cavitation nucleus" since it acts as a site for bubble growth (analogous to nuclei used for promoting crystal growth from solutions).

Another mechanism for stabilizing such gas nuclei was proposed by Fox and Herzfeld (32), and seems especially reasonable for biological solutions or suspensions. In a solution of organic molecules these will collect at any air interface which exists. The proposal is that these molecules may form a sturdy "skin" on the surface of a microbubble, which prevents diffusion and thus stabilizes it against dissolution.

3.10 BIOLOGICAL EFFECTS OF ULTRASONICALLY GENERATED CAVITATION

There have been many investigations of changes brought about by ultrasound in solutions of biological cells or macromolecules. Extensive reviews are available from El'piner (1) and from Reid and Sikov (2). In nearly all situtations where the mechanism has been identified, and has been shown to be nonthermal, it has proved to be some form of stable or transient cavitation occurring in the solution. Cavitation in biological systems has been the subject of recent reviews (33, 22).

There are many examples of chemical reactions in aqueous media which are promoted by ultrasonic cavitation. Often these are mediated by the free radicals H and OH; the latter can be produced by dissociation of H_2O vapor in a bubble during a contraction if the temperature becomes sufficiently high. Saksena and Nyborg (34) made use of available thermodynamic data to calculate the dissociation ratio δ' (number of H or OH radicals divided by the number of H_2O molecules at equilibrium) at various Kelvin temperatures T, obtaining

$$\log \delta' = 5.0 - 0.75 \log T - 12,800\, T^{-1}, \qquad (20)$$

where "log" means "logarithm to base 10." For example, at T = 2000°K the ratio δ' is about 10^{-4}. To achieve this temperature in an adiabatic compression the bubble radius must decrease by a factor of 4.8.

Theoretical plots of radius versus time for a bubble executing large oscillations show that the minima of radius (corresponding to elevated temperatures) are sharp, approaching cusps, while the maxima of radius (corresponding to reduced temperature) are broad. See, e.g., computations by Lauterborn (35). Thus the gas in such a bubble experiences a series of short pulses of high temperature; presumably the free radicals are formed during these

pulses. Unfortunately theoretical predictions have not yet been made in a form convenient for predicting the driving pressure amplitude required to achieve a given temperature elevation.

Free radicals are highly reactive and, once they are formed in a temperature pulse, proceed to combine with other species. Reactions either occur in the gas phase, at the gas-liquid interface, or in the liquid. Reviews of the many chemical reactions promoted by ultrasonic cavitation are available (1, 22). Of special interest are the many demonstrated reactions involving amino acids, proteins, nucleic acids and other biomolecules.

It is characteristically found that chemical reactions mediated by free radicals do not occur unless the ultrasonic intensity level exceeds some critical threshold value. If in its initial state the liquid being irradiated contains bubbles of optimum size for cavitation activity, one would expect the threshold to be at an intermediate level, and that the cavitation would begin immediately. More commonly, however, the initial bubbles are relatively small and a correspondingly high sound level is required to bring about noticeable free radical production. In this latter situation the threshold intensity is probably just that required to bring about bubble growth; the smaller the initial bubbles the higher the intensity required for growth. Maximum activity does not occur until after a time interval during which the bubble grows (by rectified diffusion and inter-bubble coalescence) to optimum size. The existence of a time delay is illustrated by experiments of Hill, et al. (36) in measurements of I_2 produced (with 1 MHz ultrasound at spatial-average intensities up to 4.5 W/cm^2) from KI solutions in the presence of $C Cl_4$. They found effective I_2 production with 10 ms pulses when these were spaced as much as 100 ms apart. However, for the same intensity during the pulse, no I_2 was detected from 1 ms pulses when these were separated by 10 ms or more. Apparently, under conditions of their experiment, a time interval between 1 ms and 10 ms was required for bubble growth to a suitable size.

If bubbles were initially present of optimum size the threshold for free radical production would presumably be that required to cause the H_2O vapor in the bubble to reach a sufficiently high temperature. However we should expect the threshold to be quite different for other effects of cavitation, which do not depend on free radical production but may nevertheless be significant biologcally. For example, during the contraction phase of transient cavitation the surface of a bubble moves toward its center with high speed, characteristically approaching the speed of sound. When the bubble is near a solid boundary high velocity gradients are established near this boundary with associated high shearing stresses. Even with stable cavitation, high stresses are developed near the boundary, as a result of small-scale acoustic streaming or microstreaming. (Section 4). These stresses have been invoked to explain the action of ultrasound in causing mechanical damage to biological cells and macromolecules in solutions. As might be expected, the threshold for damage from shearing stress varies from one kind of biological cell or molecule to another. The intensity required for mechanical damage via cavitation-induced shearing may be either greater or less than that for chemical damage via cavitation-induced free radicals.

Information on relative thresholds for differing biological effects of cavitation was obtained by Hughes and Nyborg (37) in experiments at 20 kHz. Small cylindrical holes approximately 0.25 mm in diameter and depth were drilled into a solid surface which was then immersed into the suspension of interest. The air trapped in each hole provided a gaseous body serving to promote cavitation, even at relatively low levels. The sound level was varied by adjusting the vibration amplitude of a driving transducer. With this arrangement studies were made on disruption of bacteria (measured by release of protein), on backbone

scission of DNA (measured by reduction of viscosity), on denaturation of DNA (measured by reduction in hyperchromicity) and on free-radical production (measured by determination of I_2 production in KI solution). A critical value of the amplitude ξ_c was found such that the denaturation of DNA and I_2 production occurred only for amplitudes higher than ξ_c. This was taken to be the threshold amplitude for free-radical production. Bacterial disruption and DNA scission occurred at amplitudes well below ξ_c, although these processes proceeded more rapidly at higher amplitudes. The disruption and scission were attributed to the action of shearing stresses set up near the bubbles. At low amplitudes these stresses are rather weak, but nevertheless cause significant alterations of biological systems under some conditions. At high amplitudes the stresses can be very destructive.

Particularly convincing evidence of the significance of mechanical stresses produced by cavitation at low levels comes from experiments by Rooney (38). He succeeded in carrying out studies on lysis of red cells brought about by a single air bubble driven at its resonant size of 20 kHz. (Fig. 7). Release of hemoglobin was seen at a fairly well-defined threshold level, corresponding to a radial displacement amplitude of the bubble surface equal to about one-tenth of its original radius. This level is far below that required for either free-radical production or for transient cavitation. The mechanism is believed to be shearing stresses set up by microstreaming near the bubble and will be discussed further in Section 4.

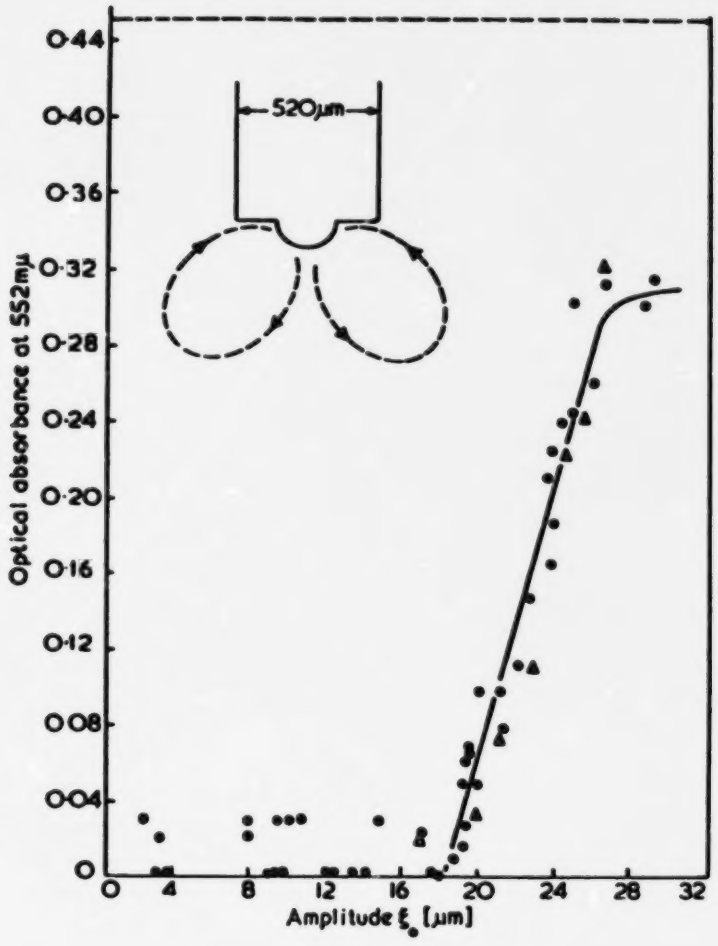

Figure 7. Hemolysis of canine red cells in the microstreaming field near a single air bubble set into stable volume oscillation at 20 kHz. Note the sharp threshold for a radial oscillation amplitude of 18 μm. From Rooney (Ref. 38).

3.11 CAVITATION IN LIVING TISSUE

The results discussed in Section 3.10 are relevant to suspensions of biological cells and macromolecules, and to body fluids. Little information exists on cavitation in bulk animal tissues; since this tissue is characteristically opaque it is usually not feasible to make direct visual observations or other optical determinations of the kind that are often made in suspensions.

Acoustical methods would seem especially promising for detecting any bubbles which might be present, and also for monitoring their activity. Since gas bubbles are very effective as reflectors or scatterers of ultrasound it is reasonable that ultrasound echo scanning would provide a sensitive means for detecting them. Mackay and coworkers have used this method with considerable

success in studying bubbles detected in mammalian tissues during decompression. In a recent paper Rubissow and Mackay (39) report routine detection of bubbles from 2 to 5 μm in diameter appearing in the blood vessels of fatty tissues in animal legs. The animals had been subjected to overpressure, and then to pressures gradually reduced to normal values. Since this decompression causes bubbles to appear it would seem that there must have been "cavitation nuclei" (probably gaseous bodies of submicron size) present initially. However, up to the present time such nuclei do not seem to have been observed directly.

Another acoustical means of detecting bubbles is suggested by the fact that, in liquids or suspensions, cavitation gives rise to emission of sound which is quite different in spectral composition from a driving sound field. Specifically, if the driving field is continuous and of single frequency f, the cavitation spectrum may contain the harmonics 2f, 3f, etc., as well as the subharmonics f/2, f/3, etc., and also components of the type 3f/2, 5f/2, etc., and noise covering a broad frequency band. It would thus seem reasonable that any cavitation produced when tissue is irradiated with ultrasound could be detected by listening devices suitable for detecting sound emitted. Lele et al. (40) have used such methods and have reported, in irradiating brain tissue with megahertz focused ultrasound, that subharmonic emission appears when the focal intensity is sufficiently high.

Evidence for the occurrence of cavitation in tissue under some circumstances comes from observations of "holes" in histological sections. Curtis (41) has reviewed reports of such holes in liver and other tissues, produced by unfocused beams in the megahertz range; these beams were generated continuously or as repeated pulses, and at intensities of only a few watts per square centimeter. But when focused beams are generated in single short pulses, the intensity required for similar occurrences is apparently much higher; Fry, et al. (42) cite a threshold value of several thousand watts per square centimeter for hole production in brain tissue by single pulses of the order of milliseconds in duration.

In plant tissues there seems to be little doubt that cavitation is significant. When such tissues are sonated at low or moderate levels and changes are found in cellular activites, or in structures, stable cavitation is probably involved. Lehmann, Herrick and Krusen (43) gave evidence of cavitation in ultrasonated plant tissue, while Harvey, Harvey and Loomis (44) pointed out earlier the significance of intercellular gas spaces. Recently Gershoy, Miller and Nyborg (45, 46) have considered the gas spaces in more detail; it appears from their work that pulsation of the spaces (i.e., stable cavitation) leads to movements and structural damage in adjoining cells. It seems likely that the chromosome damage (47) and reduction in growth rate (48) which have been reported for sonated plant systems are, at least, in part a result of this kind of cavitation. Since these occur at relatively low intensity levels it is obviously important to know if and when similar events would occur in mammalian tissue.

4. VISCOUS STRESSES AND OTHER MECHANISMS

4.1 THE ACOUSTIC BOUNDARY LAYER

When a sound field is set up in a liquid the particles of liquid take part in oscillatory flow. Suppose the liquid region under consideration is near a rigid boundary and that the latter imposes a nonslip condition on the flow. Define a Cartesian coordinate system (x,y,z) such that z is directed outward from the boundary, the latter being at $z = 0$. In the fluid all components of the velocity are zero at $z = 0$, but the x and y components characteristically increase rapidly with increasing z. For example, the x component u at any distance z from the boundary may be given (49) by

$$u = u_0 \left(\cos \omega t - e^{-\beta z} \cos (\omega t - \beta z)\right), \qquad (21a)$$

where

$$\beta^{-1} = (2\eta/\omega\rho_0)^{1/2}; \qquad (21b)$$

here u_0 is a constant and ω is the angular frequency of the sound field (assumed continuous and single-frequency) while ρ_0 and η are, respectively, the density and shear viscosity coefficient for the liquid. Examining Eqs. (21) we see that the velocity u is zero at $z = 0$ (as required) and increases to approach a limiting value, namely, $u_0 \cos \omega t$, when $z >> \beta^{-1}$ (i.e., when $\beta z >> 1$). Thus $u_0 \cos \omega t$ is a "free field" velocity and can be thought of as the velocity which would exist in the region under consideration if the restraining boundary were absent. The quantity β^{-1} has the dimensions of length. While z increases from zero to β^{-1} the factor $e^{-\beta z}$ (see Eq. (21a), decreases from 1 to $e^{-1} = 0.37$. Hence the distance β^{-1} is such that the transition in velocity amplitude from zero to u_0 occurs primarily in a layer of thickness β^{-1} near the boundary. This layer is called the acoustic boundary layer and β^{-1} the acoustic boundary layer thickness. At megahertz frequencies in water-like media this boundary layer thickness is very small; for example, at 1 MHz in water β^{-1} is approximately 0.56 μm. At the boundary $z = 0$ the velocity gradient varies sinusoidally in time with amplitude given by

$$|\partial u/\partial z| = \beta u_0. \qquad (22)$$

Thus the velocity gradient amplitude at the boundary is just equal to the free field velocity amplitude u_0 divided by the boundary layer thickness β^{-1}. To calculate a representative value of βu_0 we consider a traveling plane wave of intensity 1.0 W/cm^2 in water; then u_0 is 11.5 cm/sec. Considering this wave to be of frequency 1 MHz and to graze a rigid boundary at $z = 0$ we obtain 2×10^5 sec^{-1} for βu_0, the velocity gradient amplitude at $z = 0$. This high value is typical in the boundary layer region, but at distances more than a few microns from the boundary the gradient is negligible.

The viscous stress exerted on the boundary is just the product of the velocity gradient and the coefficient η of shear viscosity. Hence the stress at the boundary varies sinusoidally in time with frequency ω; its amplitude from Eqs. (21b) and (22), is given by

$$|S|_0 = \eta\beta u_0 = u_0 \ (\omega\eta\rho_0/2)^{1/2}. \qquad (23)$$

For the situation considered before of a plane traveling wave in water at frequency 1 MHz and intensity 1 W/cm^2, the stress amplitude is 2040 dyn/cm^2. Viscous stresses approaching this magnitude prevail throughout the boundary layer region and would act on any biomolecules or small cells which might exist there.

If such a stress were to be applied steadily, it could be a source of biological damage. However it acts in a given direction only for half a sonic period (0.5 μs in the example considered) and then is reversed in direction during the next half-period. Because it has so little time to act in each half-cycle the oscillatory shear which exists in the boundary layer is, in itself, probably of little consequence biologically. However, in many situations a sound field which generates oscillatory shear in boundary layers also gives rise to small-scale steady fluid motions called acoustic streaming or microstreaming. These are important biologically, and are taken up in the next section.

4.2 ACOUSTIC MICROSTREAMING

It was stated in Section 3.5 that time-independent eddying motion is set up near an oscillating gaseous body, or bubble, when it rests on a solid boundary, or when its surface motion lacks spherical symmetry for other reasons. This is an example of microstreaming; for a bubble resting on a boundary it arises because of boundary layers set up nearby, through interaction of oscillatory fluid motion (caused by the bubble pulsation) with the boundary, as in the example discussed in Section 4.1. The microstreaming itself is also characterized by a boundary layer. Thus the streaming velocity U is zero at a rigid boundary, say, at $z = 0$, then increases rapidly with distance z from the boundary through a small distance δ. The layer of liquid within a distance δ of the plane $z = 0$ is the acoustic streaming boundary layer, in which the velocity gradient $\partial U/\partial z$ is especially high. It is typically found that δ is of the same order of magnitude as the acoustic boundary-layer thickness β^{-1} defined in Eq. (21b). It is apparently because of the time-independent shearing stresses set up in the boundary layer that bioeffects of acoustic microstreaming come about.

While the oscillating bubble is very likely the most important source of microstreaming in many ultrasonic applications, it should be realized that the phenomenon of small-scale acoustic streaming occurs rather widely. Near any object in oscillatory motion relative to a surrounding fluid an acoustic boundary layer is established (Section 4.1). It is shown in acoustical theory (50) that if the boundary layer is nonuniform (i.e., varying from one point to another along the surface with which it is associated) a distribution of time-independent "effective force" is established. In a liquid this quasi-force leads to the circulatory fluid motion, or eddying, known as acoustic streaming. If the scale of the nonuniformity is small, as it would be near a small vibrating object, the eddies may be microscopic in size, and hence reasonably called "microstreaming."

An acoustic streaming situation which has probably received more theoretical attention than any other is that of the rigid infinite cylinder, vibrating transversely with respect to its own axis. This topic was treated by Schlichting, and by groups at the University of Oslo (Holtzmark, et al.), Brown University (Westervelt et al.), and the Massachusetts Institute of Technology (Ingard, et al.); a review appears in Reference 50. Streaming occurs in four symmetrical counter-rotating circulations as shown in figure 8. For a cylinder of radius R_0 vibrating with angular frequency ω and displacement amplitude ξ_0 the velocity gradient G at the boundary is given by

$$G = \omega\beta\xi_0^2/R_0, \qquad (24)$$

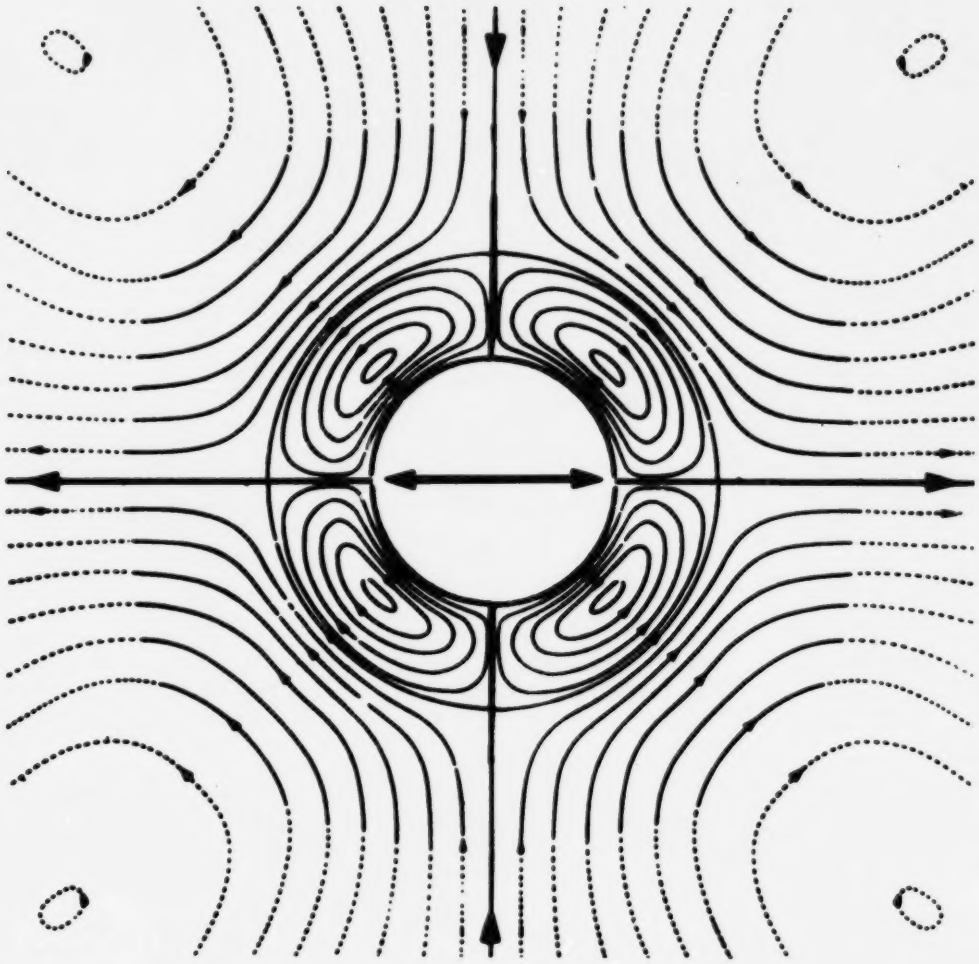

Figure 8. Acoustic streaming near a vibrating cylinder. Double headed arrow at center shows direction of cylinder oscillation. Other arrows show direction of steady fluid flow. From Holtzmark and others, as discussed in Ref. 50.

where β is as defined in Eq. (21b). This result provides theoretical basis for a vibrating-wire method first used by Williams, Hughes and Nyborg (51) for subjecting biological cells to the effects of microstreaming; see Section 4.4. It is to be expected that acoustic streaming should be important biologically because of the velocity gradients and associated shearing stresses which exist in boundary layer regions. These stresses are comparable in magnitude to those shown in purely hydrodynamical situations to cause biological damage.

In fact, hydrodynamic shearing generated by acoustic microstreaming has proved to be an important mechanism for bioeffects of ultrasound. To provide background on this topic we first, in Section 4.3, review briefly information

which is available from purely hydrodynamic (i.e., nonacoustic) applications of fluid shearing. Results obtained by special ultrasonic arrangements, operating in the 20-100 kHz frequency range, are taken up in Section 4.4. Speculations on possible implications for bioeffects of ultrasound at megahertz frequencies are advanced in Section 4.5

4.3 EFFECTS OF HYDRODYNAMICALLY PRODUCED VISCOUS STRESSES

No adequate analysis exists to show just how hydrodynamic stress acts on biological cells. To gain some insight we consider a model situation. Suppose a liquid partakes in simple shearing flow; specifically, suppose it flows along the x direction with velocity u which is independent of x but varies linearly with y.

See figure 9. If the velocity increases from u_1 to u_2 when y increases by a distance d the velocity gradient is

$$G = \frac{u_2 - u_1}{d} \qquad (25)$$

To see how this flow might affect a suspended object we consider an "object" which is indistinguishable from the suspending liquid and, in fact, is just a volume element of the liquid. It is convenient to assume the element is in the shape of an octagonal cylinder (52) as shown in figure 9. Then, keeping in mind the viscous stresses which exist in the liquid, we find that the medium outside the element acts on the octagonal element in the directions indicated by the arrows. Thus on the "90" face the outer medium pulls to the right on the octagon, and on the 270 face it pulls to the left; these directions are as expected from the nature of the field. At the 0 and 180 faces the forces are as indicated by virtue of symmetry of the stress tensor or, stated differently, the forces are in these directions in order to balance torques on the element. By considering the stress tensor in axes rotated through 45° one finds that the forces on the 45, 135, 225 and 315 faces are normal to the faces and are in the directions indicated. It can be seen that a net effect of the stresses acting on all the faces is to provide a tendency to stretch the element along the 45 and 225 directions, while compressing it along the 135 and 315 faces.

Of course the element is usually under positive hydrostatic pressure. This would be represented on the figure by equal forces acting inward on all faces, but can be ignored for our present purposes. In addition to the deformation expected from the forces shown on figure 9 the volume element will rotate clockwise as a result of the steady shearing flow.

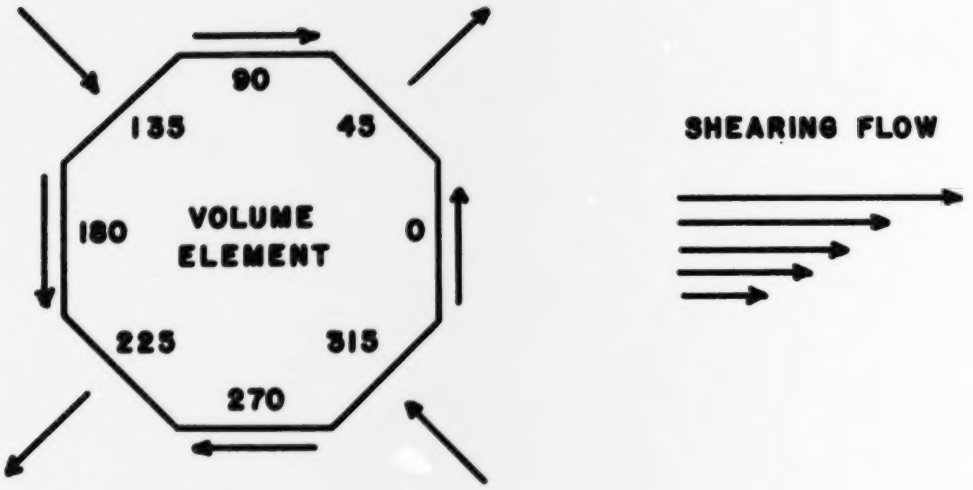

Figure 9. A volume element of fluid, octagonal in shape, taking part in simple shearing flow. From Ref. 16.

When a foreign object is introduced in the flowing liquid, the liquid flow path and the associated stress distribution will be somewhat altered. Also the object will, in general, not respond to the stresses in the same way as an element of the liquid itself, especially because of constraints resulting from elasticity and/or surface tension. It would be expected, however, that if the object and the liquid are not too dissimilar there should be some correspondence in behavior between the object and the fluid element. Investigations carried out at McGill University by S.G. Mason and associates (53) are informative on this matter. In some of their work they have made observations through a microscope on droplets of a given fluid, suspended in another fluid which is undergoing shearing flow. An example of their findings is shown in figure 10 where the sketches "1", "2", "3", etc., represent the appearance of a droplet at increasing values of time after the shearing began. In this sequence, the droplet begins as a sphere in "1", and becomes increasingly elongated in "2" and "3"; this elongation and the obvious rotation are as expected from our previous discussion of the volume element. In "4" the length/width ratio exceeds a critical value (about 3) and thinning occurs at the center. With continued shearing the two ends of the droplet separate (in "5") and, in this example, a number of very small droplets form in addition to the two larger ones. This droplet formation process is, in a general way, as expected from theory given by Rayleigh (54); he showed that a thin cylinder of fluid, whose surface energy is high relative to an outer fluid, is unstable and may become fragmented.

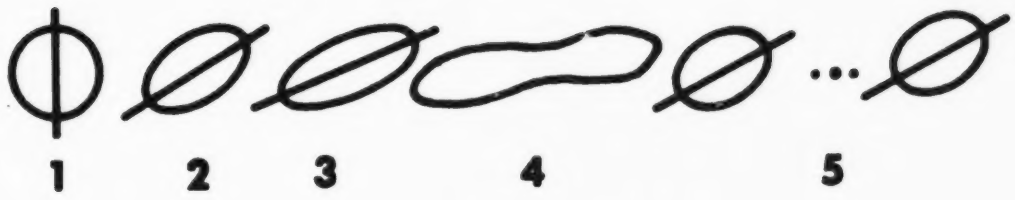

Figure 10 Distortion of a suspended droplet in flow with a velocity gradient. Velocity horizontal to the right, increasing vertically. Droplet initially spherical, becomes elongate and may subdivide. From F.D. Rumscheidt and S.G. Mason, J. Colloid Sci. 16, 238-261 (1961).

In experiments with biological cells in shearing fields a favorite test object is the canine or human red cell. A review of findings has been given by Rooney (55). While the results are somewhat related to those obtained with droplets, an obvious difference exists since the red cell has a membrane. A very common consequence of shearing is the separation from the red cell of many small spherical fragments of diameter 0.5 to 2.0 μm, referred to as microspheres. See Nevaril, et al. (56) and Champion, et al. (57). A number of groups have found that when the shearing stress exceeds a critical value S_c hemolysis occurs, that is, hemoglobin begins to leak from the cells. Results were summarized by Leverett, et al. (58). They point out the importance of the time during which shearing is applied to the cells. Reported values of S_c vary from a low value of 1500 dyn/cm^2 for a shearing time of 100 sec to a high value of 40,000 dyn/cm^2 when the time is only 10^{-5} sec. Williams (59) has suggested an explanation of the time dependence in terms of viscoelasticity of the red cell membrane.

It was also found, by Krizan and Williams (60) that S_c depends critically on the temperature. Some of their findings are shown in figure 11 where the experimental points are for human erthrocytes subjected to simple shear for five minutes. While S_c is about 600 dyn/cm^2 at 25° C it decreases with increasing temperature, dropping essentially to zero at 48° C. The solid curve plotted on the same figure is calculated from theory based on a model from statistical mechanics for phase transitions. The fit between theory and experiment is excellent and gives strong support to the idea that hemolysis is correlated with some kind of phase change in the membrane. Nanjappa, et al. (61) reported other changes in canine red cells produced by shearing stresses as low as 90 dyn/cm^2. (Such a low stress is comparable to shearing stresses encountered in the natural circulation.) These changes include reduction in half-life of the red cell (after shearing for 5 min), increased susceptibility to osmotic lysis (after 15 min of shearing), altered permeability and loss of surface enzymatic motility (the latter both after 60 min.).

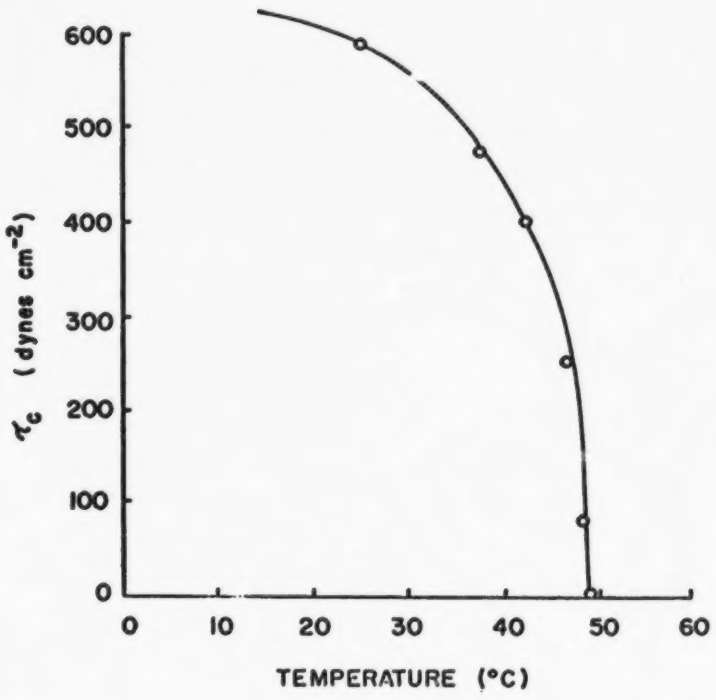

Figure 11. Critical shear stress S_c for erythrocytes as a function of temperature during shear. Circles are for experimental data while curve is plotted from physical theory for a phase transition. From Krizan and Williams (Reference 60).

In work with human platelets Brown, et al. (62) showed that hydrodynamic stresses of about 150-250 dyn/cm^2 applied for 5 min or less affect the platelets by causing release of acid phosphatase and serotonin, and also by causing platelet aggregation.

Up to this point in discussing effects of shearing we have dealt only with droplets and biological cells. It is an important finding that hydrodynamically produced shearing stresses can also cause damage to large biomolecules. This was demonstrated in dramatic fashion by Levinthal and Davidson (63) who showed that ordinary pipetting procedures generate enough shearing stress to reduce the molecular weight of DNA from an initial very high value to a much smaller one. They derived an approximate equation for predicting the shearing stress S_c required to produce a break in a rod-like molecule:

$$S_c = \frac{16\ F\ \ell n\ (L/a)}{3\pi L^2} \qquad (26)$$

Here "ℓn" means "natural logarithm," L is the length of the rod, a its radius and F its breaking strength. On the basis of molecular bond strengths in the

two strands of DNA the authors calculated F to be about 0.0016 dyn. For a molecule 65 μm long and 4 nm in radius one obtains from Eq. (26) that S_c is about 180 dyn/cm^2. Of special interest is the dependence of S_c on L, according to Eq. (26); since the logarithm is a slowly varying function, the length dependence is determined primarily by the L^2 factor in the denominator. Therefore, according to Eq. (26), long thin molecules are much more susceptible to shear than short thick ones of the same molecular weight.

4.4 BIOEFFECTS OF ACOUSTIC MICROSTREAMING

In view of the findings for hydrodynamic viscous stress discussed in Section 4.3, it is not suprising that ultrasound affects living systems by means of the small scale flows which are set up near inhomogeneities. As anticipated in Section 3.5 the gas bubble, set into pulsation by an ultrasonic field, is especially significant as a source of microstreaming. From approximate theory for a bubble resting on a rigid boundary (50) it follows that the velocity gradient G at the boundary can be expressed just as in Eq. (24); letting S be the viscous stress ηG we have

$$S = \omega n \beta \xi_0^2 / R_0; \qquad (27)$$

here ω, as before, is the angular frequency and β is given by Eq. (21b); however, R_0 is now the radius of the bubble (in the absence of pulsation) and ξ_0 is the radial displacement amplitude of its surface.

Rooney (38) was able to carry out experiments at a frequency of 20 kHz, in which canine red cells were subjected to the action of a single pulsating gas bubble of resonant size (R_0 = 125 μm). See figure 7. Hemolysis was assayed by optical measurements of the quantity of hemoglobin released. To stabilize bubble motion against surface waves the cells were suspended in a Dextran-saline solution whose viscosity (measured with a capillary viscometer) was about thirty times that of water. Little hemoglobin was detected until the amplitude ξ_0 exceeded a rather definite threshold. From Eq. (27) the viscous stress S corresponding to this amplitude is 4300 dyn/cm^2. Because the velocity gradient is nonuniform in the microstreaming situation, there is uncertainty (estimated to be 1500 dyn/cm^2 or less) in the value of S.

As we stated earlier, when steady hydrodynamic shearing is applied to red cells for 100 sec the stress required to produce noticeable hemoglobin release is only 1500 dyn/cm^2. This is significantly less than the threshold value quoted by Rooney for bubble-associated microstreaming, a fact which has been reasonably explained in terms of a time factor. In the microstreaming situation the stress is applied to a given cell only for a very short length of time, while the cell is passing through a boundary-layer region of microscopic size, in the vicinity of the bubble. Hence, although the sound was applied for five minutes in Rooney's experiment, each individual cell may have been subject to stress of significant magnitude for only a few milliseconds. Because of this, and since the stress threshold is inversely related to time of stress application, it is reasonable that in microstreaming situations this threshold is higher than in other flow fields where stress is applied to cells steadily for many seconds or minutes.

At amplitudes above the threshold value the rate of Hb release increases rapidly. The rate apparently depends partly on the number of cells brought into the critical boundary layer region per unit time, and partly on the stress applied to each cell as it passes through the critical region. Both of these factors increase with the streaming velocity U which, in turn, increases with the amplitude ξ_0; under simple conditions U is proportional to ξ_0^2.

Support for the explanation of Rooney's results in terms of microstreaming theory comes from experiments by Williams, Hughes and Nyborg (51). These investigators used a tungsten wire of 125 μm radius vibrating transversely at 20 kHz to generate microstreaming. Theory for acoustic streaming near a vibrating cylinder was applied; from this came Eq. (24) which gives the velocity gradient G at the boundary. Using this result for G, and letting the stress at the surface be $S = \eta G$, we obtain an expression for S which has the same form as that given in Eq. (27); however, ξ_0 and R_0 are now parameters of the cylindrical wire, as defined in connection with Eq. (24). When experiments were done with red cells in Dextran-saline solutions under conditions similar to Rooney's, the results were also similar. Thus release of hemoglobin was observable for amplitudes above a certain threshold ξ_0; furthermore the absolute value of the threshold amplitude was about the same as for the bubble. Since all other parameters in the expression for S (radius, frequency, viscosity, etc.) were nearly the same in the two experiments it follows that the calculated values of critical stress for the vibrating-wire experiment agreed well with value from experiments with the vibrating bubble. The vibrating wire has been used further by Williams in studying bioeffects on other cells and also in studies of DNA fragmentation (64). Of special interest are his results showing serotonin release from human platelets (65). The shearing stress required to modify the platelets in this way was of the order of ten times less than the stress required for release of hemoglobin from red cells. This difference in sensitivity to shearing stress of platelets as compared to red cells is as found by Brown, et al. (62) using nonacoustic methods.

4.5 SPECULATION ON MICROSTREAMING AT MEGAHERTZ FREQUENCIES

It is important to know whether stresses of the magnitudes referred to in Section 4.4 can occur in the field of such devices as are used in medical ultrasound. In approaching this question it would be helpful to know at what intensity level biologically effective stresses can be generated in a megahertz frequency range. No experimental information seems to be available on this matter, so we shall attempt a rough estimate by using theory to relate findings at a frequency of 20 kHz to expectations at higher frequencies. To estimate the lowest intensity at which significant stresses can be generated, we consider the situation of a pulsating bubble resting on a rigid boundary. Equation (27) is then applicable; it can be reorganized to yield

$$\frac{\xi_o}{R_o} = \left[\frac{S}{\omega R_o}\right]^{1/2} \left[\frac{2}{\rho\omega\eta}\right]^{1/4} \qquad (28)$$

This gives the radial displacement amplitude ξ_0 required to yield the stress S just outside the bubble and near the boundary on which it rests. Suppose the bubble exists in blood, whose viscosity in the circulation has been estimated to be 0.04 P. If the frequency is 1 MHz and the bubble is resonant at this frequency we take R_0 to be 3 μm. Suppose the stress required for damage is 800 dyn/cm^2; this is intermediate between quoted values for red cells and those for platelets. Setting S equal to this value we obtain for the relative amplitude ξ_0/R_0 the value of 0.035. This small value for ξ_0/R_0 means that the bubble oscillation can occur in a range where the linear approximation holds; it is not necessary to deal with the complications of nonlinear cavitation phenomena. We can now proceed to estimate the pressure amplitude p_0 required in an exciting wave to produce this oscillation amplitude. From Eqs. (15) we obtain, for a resonant bubble.

$$\frac{p_o}{P_o} = 3\gamma\delta \frac{\xi_o}{R_o} . \qquad (29)$$

For purposes of a rough estimate we let γ be 1.4. Its exact value is not known; it should be smaller than 1.4 but not less than 1. Letting δ be 0.14 (indicated earlier as appropriate for water) and taking the previously calculated value 0.035 for ξ_0/R_0 we obtain 0.04 atm for p_0. In a plane travelling wave the intensity corresponding to this pressure amplitude is 0.5 mW/cm^2. Hence the kind of reasoning we have followed in this section has lead us to the conclusion that a significant biological effect might be obtained in a field of intensity less than 1 mW/cm^2, provided a bubble of resonant size is present, in the vicinity of a rigid boundary.

It is startling to find possibilities for bioeffects at such low intensity levels. It is also puzzling, since the lowest levels at which biological effects have been reliably reported are considerably higher than this. There are a number of reasons which might be considered for the discrepancy:

(1) The theory presented here involves a number of approximations and extrapolations. Neither the analysis for bubble dynamics nor that for acoustic streaming have been tested at mehahertz frequencies.

(2) Perhaps bubbles resonant to megahertz frequencies and situated near a suitable boundary do not exist in experimental situations at low levels.

(3) The scale of distance for the expected microstreaming at 1 MHz is small; because of this a given magnitude for the stress may be less effective than at low frequencies.

(4) Perhaps changes do occur but have not been detected.

This topic will be referred to again in Section 5.

4.6 RADIATION FORCE

Consider a sound field set up in a liquid. If droplets of another liquid, or solid bodies or other inhomogeneities exist in the liquid, forces will be exerted on these and also on the container walls, because of the sound. In part, the force will be oscillatory in time, with the same frequency as the sound; the time-average of this part is zero; but another component of force is usually present also, whose time-average is not zero. This time-independent or steady component is called the radiation force. An important application of radiation force is in measuring the output power or intensity for an ultrasonic device. Here the beam is usually caused to interact with a plane reflector, a plane absorber or a solid sphere; the force is measured and the intensity is computed by means of available theory.

It is reasonable that radiation forces should be important also when ultrasound interacts with biomaterials. In a suspension such as blood the solute particles would tend to be driven by radiation forces into new distribution patterns. This may explain the stasis phenomenon reported by Dyson, Woodward and Pond (66); these investigators have shown that red cells (in blood vessels of chick embryo) collect into parallel bands spaced one-half wavelength apart in an ultrasonic standing wave field (Fig.12). Gould and Coakley (67) have investigated the situation in vitro; they conclude that radiation forces are involved, but that there are complications not accounted for by simple theory.

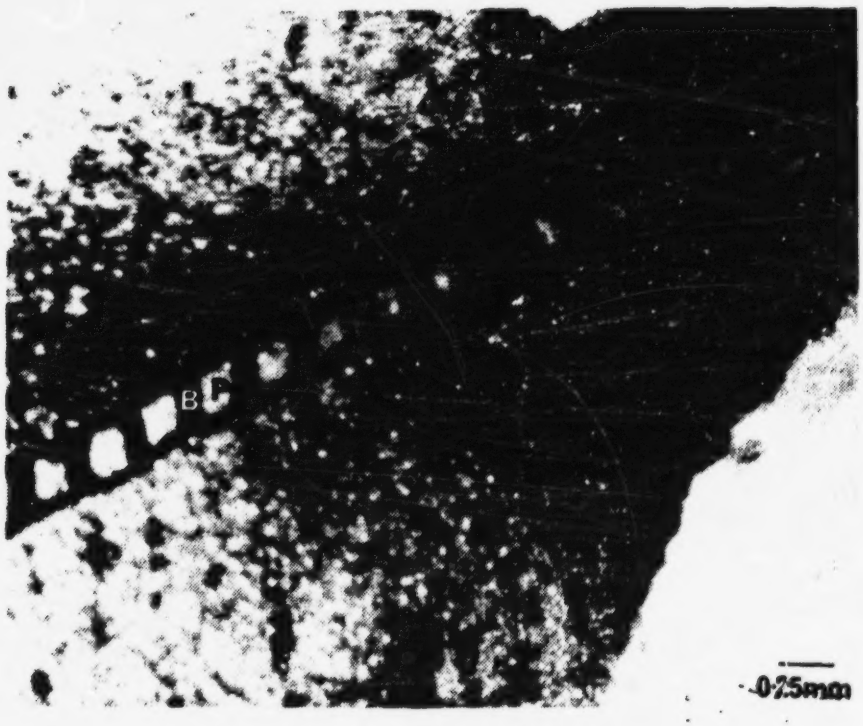

Figure 12. Ultrasonically induced stasis of red cells in blood vessel of chick embryo. Spacing between bands of red cells is one-half the sonic wavelength. Dyson, Woodward and Pond (Ref. 66).

If a particle suspension is rather dilute, so that the particles are widely spaced, the radiation force on each particle is as if the others were absent. However, if the particles are closely spaced they interact with each other by virtue of interparticle radiation force. The theory for this force has been discussed by Gershoy and Nyborg (68) and in a review (69); it is closely related to theory given much earlier by Konig (70) and Rayleigh (71). One interesting result applies to a pair of small particles whose separation is small: the sign of the radial component of the interparticle force depends on the orientation of the particle-particle (pp) axis relative to the local direction of the fluid oscillation. Specifically, if the (pp) axis and the oscillation direction are perpendicular the force is attractive and the particles tend to coalesce; if those directions are parallel the force is repulsive. This feature of the force leads to the formation of particle chains in suspensions (Nyborg, Miller and Gershoy, (72)); these are similar to the "pearl chains" reported in microwave fields (Herrick, (73)). See figure 13.

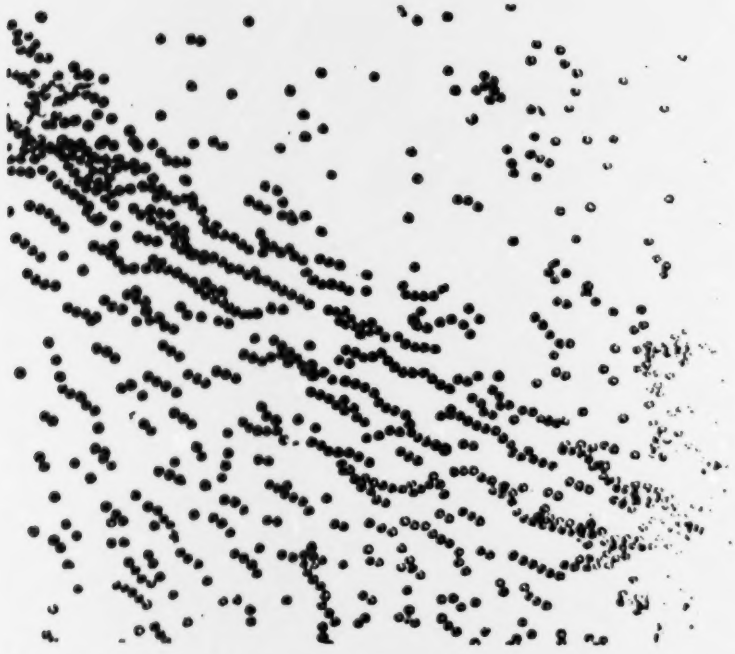

Figure 13. Pearl-chain formation in a 1 MHz ultrasonic field; the particles are sphered erythrocytes. From Nyborg, Miller and Gershoy. (Reference 72).

The radiation forces exerted on particles can be explained, in part, by the fact that a sound field in a fluid medium produces, in effect, an excess (positive or negative) static pressure which varies from point to point in the field. We shall call the excess static pressure at any given point the radiation pressure there. Gradients in the radiation pressure contribute to the radiation forces on suspended bodies. Also, if small tubes exist in the fluid, flow may be induced in these tubes; a difference in radiation pressure at the ends of a tube will cause the fluid within it to flow. Gershoy and Nyborg (74) have invoked this idea to explain flow produced by ultrasound in the cytoplasmic layer surrounding the large vacuole of a plant cell. In their explanation a radiation pressure field is set up in the vacuole; the "tubes" are liquid-filled spaces between membranes of endoplasmic reticulum, and other solids, in the cytoplasmic layer.

4.7 RADIATION TORQUE

When a sound field is set up in a liquid a twisting action may be exerted on any suspended object, and also on elements of the fluid itself. This sonic torque has an oscillatory component, whose time-average is zero. In addition there is a time-independent or steady contribution, the radiation torque. For an asymmetric object, such as a disk or a rod, the torque varies with orientation of the object relative to the oscillation direction of the surrounding liquid, and the object tends to assume a favored orientation. This orienting effect may be important in

connection with effects of ultrasound on asymmetric cells, organelles or macromolecules.

When a freely suspended spherical body is acted on by radiation torque it is set into steady spinning motion. According to theory, spinning is expected in fields such as exist at a boundary where a progressive wave impinges obliquely and is reflected. The spinning velocity is then proportional to α/η, where α is the absorption coefficient for the material in the spherical body, and η is the coefficient of shear viscosity for the surrounding fluid (69). A number of investigators have observed spinning of intracellular bodies during sonation. See Nyborg, Miller and Gershoy (72). Particularly significant biologically are the findings of Dyer (75) that when the boundary of moss protonema is vibrated during the process of cell division the incipient cross-wall is set into steady rotation about an axis perpendicular to the protonemal axis. See figure 14. After sonication he found that the cell division proceeded in a number of instances, but that the daughter cells and their progeny were abnormal, the abnormality persisting throughout a series of cultures.

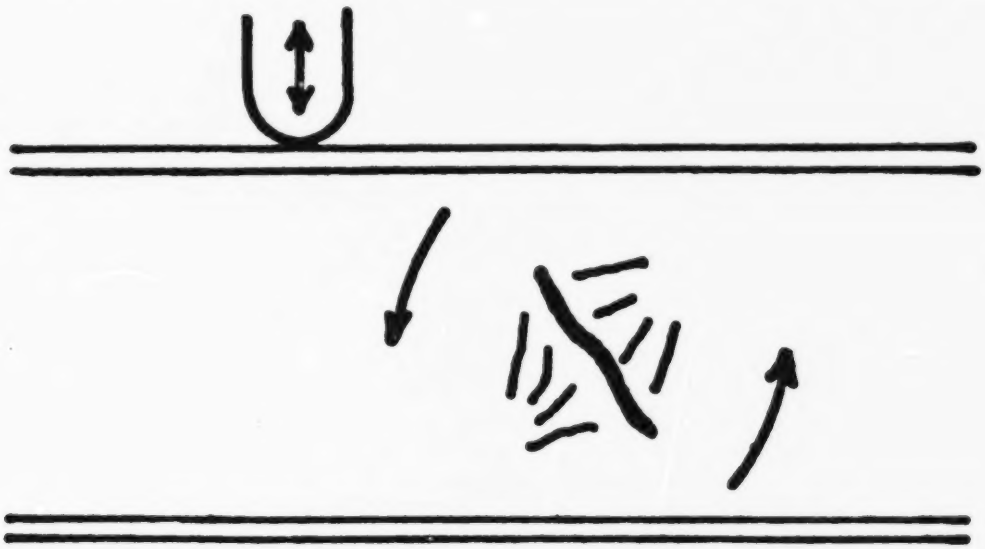

Figure 14. Rotation of incipient wall in moss protonema as observed by Dyer; discussed in Reference 72.

4.8 RESPONSE OF A SEMI-SOLID MEDIUM

Much of the foregoing discussion of mechanisms for bioeffects is based on the assumption that the biological medium is a liquid or a suspension. (An exception is the treatment of thermal effects in Section 2.) This liquid model is surprisingly useful, even for media which are not fluid. For example, it appears from Storm's experiments (28) that even cavitation, a phenomenon which might seem peculiar to liquids, actually occurs in a "soft solid" medium (gel); some characteristics of cavitation in the gel were found to be similar to those which obtain in a liquid. However, it is obvious that a liquid model is inadequate for biomedia, and that solid-like properties of tissues should be taken into account. At present there has been little done in accounting for

changes produced by ultrasound in tissues by models for solids. However, Lele, Senapati and Hsu (76), as well as Dunn, Lohnes and Fry (77) have suggested that fatigue may play a role when ultrasound interacts with brain tissue. Dunn, et. al., base their suggestion on data for the lowest intensity level I_{min} at which a lesion is detected in mammalian brain when the latter is subjected to a pulse of focused ultrasound, Letting N be the number of sonic cycles during a pulse they found that for any given frequency f the plot of log I_{min} versus log N is a straight line with negative slope one-half. Thus $I_{min}\sqrt{N}$ is equal to a constant (C) for given f. They found further that C is not strongly dependent on f. If C were completely independent of frequency it would mean that ultrasound of given intensity produces a lesion in brain after a given number of cycles, whatever the frequency. The situation is then analogous to that for fatigue of solid bars or other structures as a result of periodic flexing. It is commonly found that, for a given amplitude ξ of flexing, failure occurs after a given number N of cycles, regardless of frequency. (Also plots of log ξ amplitude versus log N for metal fatigue are often found to be linear, by analogy to the corresponding plots for brain lesions.) When an observed change occurs after a fixed number of cycles, regardless of frequency, it suggests the following:

i) A certain amount of "damage" is done during each cycle; and

ii) The damage accumulates cycle by cycle until the total reaches a level required for the observed change which may be fracture of a solid, or lesion formation in a brain.

Whether or not this concept is supported by further studies, questions remain as to the basic prosess(es) leading to the damage.

An interesting theory was put forward by Welkowitz (78) for explaining certain bioeffects of ultrasound on frog tissue, specifically, paralysis of hind legs and alteration of cell potentials in muscle. He considered unidirectional forces (presumably, radiation forces) set up by the sound on particles or systems of particles in the tissue. While the theory is couched in rather general terms, Welkowitz assumed (i) that the force on each particle is proportional to the square of the sonic amplitude, (ii) that the particle is displaced from its equilibrium position a distance proportional to the force and (iii) that the bioeffect of interest occurs when the particle displacement reaches or exceeds a critical threshold value. He derived a theoretical expression which fits available data rather well (on threshold amplitude versus duration of sonic irradiation) at low amplitudes but, as pointed out by Dunn (79), not at higher amplitudes.

Schnitzler (80) sonated muscle tissue under conditions where effects of temperature elevation and cavitation could be ruled out. Using a microvibrator with a small tip vibrating at a frequency of 85 kHz, he was able to apply ultrasound to selected portions of individual muscle cells. Changes in cellular ultrastructure were brought about, which were identified and characterized by electron microscopy. For example, mitochondrial cristae and portions of the sarcotubular system near the point of sonication (within a few tens of microns from it) were distorted or disrupted at relatively low amplitudes. Schnitzler attributed the structural perturbations to sonically generated stresses such as those which would, in a liquid, give rise to microstreaming. (See Section 4.2.)

Frost (81) has carried out model experiments with viscoelastic solids which would seem to give insight on effects of ultrasound on soft tissue. In his experiments strain-birefringent epoxy was used as the test medium. In each test a small rectangular plate of the plastic was positioned on the stage of a polarizing microscrope. Ultrasonic vibration of frequency 80-90 kHz was applied locally at an edge by means of a microvibrator positioned with a

micromanipulator. Information on the spatial distribution of strain (especially, the time-average strain) set up in the medium was obtained by observing and photographing photoelastic fringes. The microvibrator was pressed into contact with the test medium by means of a steady force F_s applied normally to the edge. Results were found to be very dependent on the value of F_s, and on the curvature of the microvibrator tip. At relatively low amplitudes the action of the ultrasound is mechanical in nature and, specifically, is approximately equivalent to increasing the steady force to a new value $(F_a + F_s)$. Under some conditions F_a is proportional to the vibration amplitude. At higher amplitudes both thermal and mechanical effects of ultrasound intervene. Some of the results were explained in terms of a viscoelastic model involving an ultrasonically activated viscosity coefficient. Also evidence was given for a solid-solid phase transition caused by the ultrasound.

Novel history-dependent phenomena were observed by Frost, which he has called "recording" and "erasing". Under some conditions strain patterns produced in epoxy during sonic irradiation remained after the irradiation ceased and were thus recorded. By suitable re-application of ultrasound the strain patterns were removed, and thus erased.

In future studies, it seems clear that soft solids should be given much more attention than they have, as models for biological media. For example, theoretical development is called for in order to elucidate the role played in such media by time-averaged stresses, analogous to those which give rise to radiation pressure, radiation torque and acoustic streaming in liquids and suspensions. At the same time experimental studies are needed, in order to identify phenomena of interest and to provide tests for theory.

5. MINIMUM LEVELS FOR OBSERVED BIOEFFECTS

5.1 INTRODUCTION

Understanding of mechanisms for bioeffects of ultrasound is essential for the science of biological ultrasound, as well as for immediate practical purposes. As is probably evident from previous chapters, useful knowledge already exists on interactions between ultrasound and biosystems, but much remains to be learned. A need for more insight on these interactions becomes evident when attempts are made to define conditions under which human beings can be exposed to ultrasound with adequate assurance of safety.

Gaps in our knowledge are revealed in this section (Section 5) as we summarize available information on physical conditions under which ultrasound produces just-discernible changes in living systems. This topic is a critical one as we try to specify ranges of ultrasonic parameters which correspond to good practice of medical ultrasound. For this purpose there is special interest in mammalian tissues, results for which are discussed in Section 5.2. Also pertinent to the topic of "safety" are the data presented in Section 5.3 on commercial units for medical ultrasound; comparisons are then made between instrumental output levels and the levels at which bioeffects have been demonstrated. Other biosystems are taken up in Sections 5.4 and 5.5, where data are considered which come from experiments with plant tissues, and also from vitro experiments with biological suspensions. In connection with bioeffects generally, attempts are made to relate findings to results expected on the basis of the mechanisms disscussed in Sections 2, 3, and 4.

5.2 DATA FOR MAMMALIAN TISSUES

Many experiments have been carried out on small animals such as mouse, rat and rabbit. From these we select here only those which satisfy the following criteria:

a) Adequacy of physical measurements. All data are from publications (or papers presented at professional meetings) in which specifications are given of intensity levels, with explanations of how these levels were measured.

b) Relevance to the question: What are the minimum values of intensity and time at which significant bioeffects occur? If these questions could be answered we would, of course, be in a position to define with confidence the conditions for safe use of medical ultrasound.

In figure 15 is an intensity-time diagram similar to ones which have been used previously (3-5) in summarizing conditions for bioeffects of ultrasound. Each point gives the intensity and exposure time corresponding to conditions of an experiment. The frequencies vary, but are in the "megahertz range," from about 0.5 MHz to 5 MHz. For the data in this figure, the sound field is essentially that generated by a flat "pistonlike" transducer. In the "far field" it is typically characterized by a central maximum and minor side lobes. The intensity plotted as ordinate is the spatial peak (Sp) value: usually the biological test object, or or a critical part of it, is subjected to the Sp intensity. For a pistonlike field this is typically larger, by a ratio of 2 to 4, than the spatially average (Sa) value frequently quoted. (From theory for an ideal piston in a perfectly rigid baffle Sp/Sa is exactly 4. For a focused transducer Sp/Sa is usually larger than 4; on the other hand for some transducers this ratio is somewhat less than 2.) For some points in figure 15 the Sp intensity was estimated (assuming Sp/Sa = 3) from a given Sa value. For all data in this figure the intensity quoted is the "free-field" value; i.e., it was measured in water (or an acoustically similar medium) in the absence of the test animal.

Most of the data in figure 15 are for continuous (CW) ultrasound. For those which apply to repeated pulses the intensity plotted is the time-averaged (Ta) value, which is less than the temporal peak (Tp) intensity by the "duty factor," the latter being the ratio of "on" time to total time; the time plotted along the horizontal axis is the total elapsed time ("on" time plus "off" time) during which the pulsed ultrasound is applied. For example, if the typical (spatial-peak temporal-peak or SpTp) intensity during a given pulse is 10 W/cm^2 and the duty factor is 0.02 the ordinate plotted in figure 15 is 0.2 W/cm^2, the spatial-peak temporal-average or SpTa intensity. For the data in this figure, the longest (total) time is 5400 s or 1.5 hr. The shortest time is 10 seconds; in clinical practice the examination time is seldom less than this.

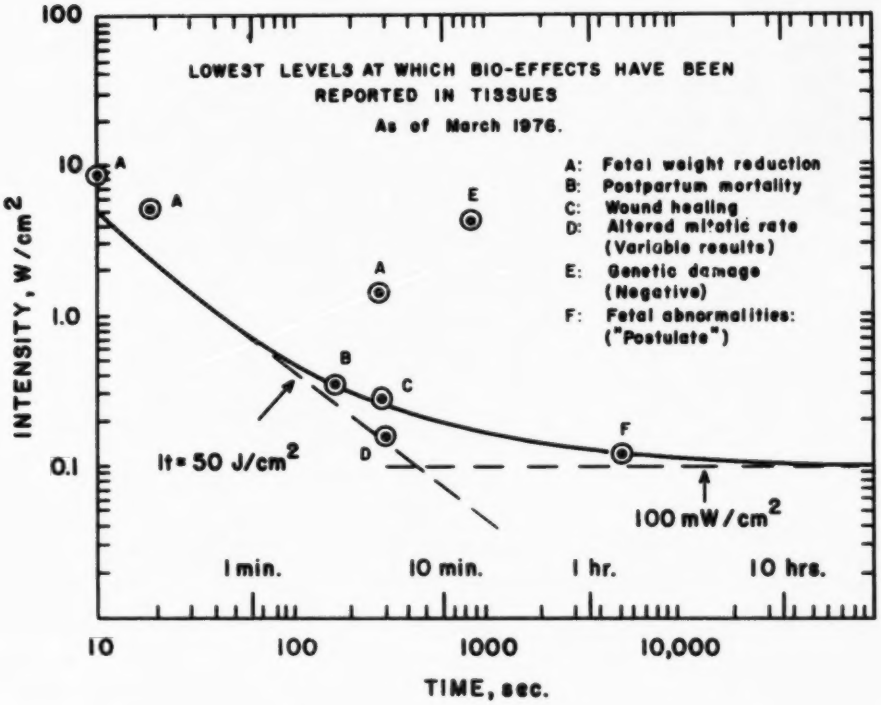

Figure 15. Summary of selected data for bioeffects of unfocussed ultrasound on mammalian tissues.

Referring now to specific experiments, the set of three points (A) are for data recently reported by O'Brien (82). In experiments done at the Bureau of Radiological Health pregnant mice were irradiated at 1 MHz under well-prescribed conditions with CW operation, and the progeny examined at about the time of birth. The mean weight per fetus was reduced significantly for the conditions represented by each of the three data points. For the lowest of these the Sp intensity was about 1 W/cm^2 and the irradiation time was 5 minutes.

Point B is also for very recent studies at the Bureau of Radiological Health, using 1 MHz continuous ultrasound. Curto (83) found irradiation of pregnant mice for three minutes at an Sp intensity of 0.35 W/cm^2 to result in significant increase of mortality in litters observed in the 21 days after birth. Point C represents findings of Dyson and co-workers (84,85) at Guy's Hospital. They investigated several related phenomena, all of which occurred at SpTa intensity levels of 0.1 to 1.0 W/cm^2 applied for time intervals in the vicinity of 5 minutes. These include the healing of surgical wounds and the stimulation of tissue repair in varicose ulcers. Results were obtained with CW irradiation, and also with 2 ms pulses separated by 8 ms "off" intervals; for a given SpTa intensity, the pulsed mode proved the more effective.

At a still lower intensity level is point D. This pertains to mitotic activity in rat liver after partial hepatectomy, the animal having been subjected for 5 min to CW ultrasound (180 mW/cm^2 Sp intensity estimated from given Sa intensity of 60 mW/cm^2) prior to the hepatectomy. Results under these conditions

have not proved reproducible: Kremkau and Witcofski (86) report positive effects on the mitotic rate, while Kaufman, Miller and Carstensen (87) report negative findings.

Negative results on genetic damage are represented by point E. Lyon and Simpson (88) exposed gonads of male and female mice for 15 minutes to pulsed and continuous ultrasound at SaTa intensities of 1.6 W/cm^2 (SpTa or Sp value of 4.8 W/cm^2 by our estimate) and observed no genetic damage. However, an increase of sterile matings was observed, in agreement with others (Sunden (89), Fahim, et al. (90)) that male sterility is induced under approximately these conditions.

Point F is for computed effects of long-term irradiation of pregnant mice. In his investigations at the Massachusetts Institute of Technology, Lele (91) has shown that a uniform intensity of 125 mW/cm^2, applied for 90 minutes will cause an increase of temperature in the uterus high enough (several degrees Celsius) to cause fetal abnormalities. He has proposed this explanation for the abnormalities reported by Shimizu and Shoji (92). While Lele has described his reasoning as postulational it appears to be based on very firm grounds. However, he has emphasized the great differences (especially in size) between mouse and man, and has argued that the ultrasonic device which causes thermal damage in mouse will be harmless in man.

In surveying these results for mammalian tissues we find that all data points in figure 15 lie within an easily defined region. This region is bounded below and to the left by a combination of two dashed curves: one slants at an angle of 45° on the log-log plot and is labeled "It = 50 J/cm^2;" the other is horizontal and represents a constant intensity level of 100 mW/cm^2. The slanted line is a locus of points for which the product of intensity I (in watts/cm^2) and time t (in seconds) is equal to 50 watt-seconds (joules) per square centimeter; here t is the total elapsed time during a treatment and, for pulsed regimes, includes both "on" and "off" time. (Some authors cite the spatial-temporal peak (SpTp) intensity for I and the total "on" time for t; the product (It) is unaltered by this pair of changes.) Thus both of the following statements apply to the data of figure 15:

> No substantial bioeffects have been demonstrated for SpTa intensities less than 100 mW/cm^2; (STATEMENT A)

and

> No substantial bioeffects have been demonstrated for which the product (It) is less than 50 J/cm^2 where, for pulsed operation, t is the total ("on" + "off") time. (STATEMENT B)

These statements apply to the data shown, which are for mammalian tissues and for exposure times from 10 s to about 100 min.

Other data might have been cited, such as those of Bender, Janes, and Herrick (93) on hemorrhage and other damage in bone marrow and those of Baum (94) and Sokollu (95) on cataracts and other changes produced in the eye. These show that bone marrow and the eye lens are sensitive to ultrasound, in that they exhibit significant effects at rather low values of intensity and time. However, even for these tissues, the data are in agreement with Statements A and B.

In fact it appears that these Statements apply to all existing reliable data on mammalian tissues in the indicated ranges of frequency and exposure

time. To test their generality this author has supplemented his own information by consulting a "Jury" of individuals who are well informed on bioeffects of ultrasound, either through their own research, or through knowledge of the literature, or both. Each individual was asked to evaluate a statement equivalent to the combined Statements A and B and, in particular, to identify any violations of it. Much useful information was revealed by this inquiry, but exceptions to the Statements were not discovered. Thus the "Jury verdict" (as of March 1976) is that these Statements apply to all existing data for significant bioeffects in mammalian tissue, subject to qualifications already noted (96).

(There are several reports of alterations in mammalian tissues produced at intensities lower than those specified in the Statements. None of these seems to provide a valid exception to the Statements, either because the physical measurements are inadequate, because the reported biological effect has not been convincingly demonstrated, or because the alteration observed has not been shown to be significant biologically.)

Suitable data are not available on bioeffects for long-term ultrasonic exposures extending beyond the range of figure 15. For effects of short single pulses (less than the 10-second lower limit of figure 15) we have the results shown in figure 16. Here line A represents some of the data reported by W.J. Fry and F. Dunn (97) at the University of Illinois, in which some very accurate measurements were made on the intensity levels at which paralysis occurs in young mice. For these the spinal cord was irradiated at several different temperatures. Line A shows the Sp intensities (I) at which paralysis occurred 50% of the time at 37° C; for irradiation times increasing from 0.1 sec to 7 sec the corresponding intensity decreased from 100 W/cm^2 to about 20 W/cm^2. (It might be remarked that it was for reasons of convenience and accuracy that the intensity corresponding to 50% paralysis was chosen for plotting in figure 16. Examination of original data shows that the intensity for, say 5% incidence of paralysis is only a little lower than the 50% value. The difference would hardly be perceptible on the log-log plot of figure 16.)

On the same figure is a dashed line with negative slope of unity on the log-log plot, representing conditions for which the product (It) is equal to 50 J/cm^2; this is an extension of the similar line which appears on figure 15. We see that the dashed line intersects Curve A at a point corresponding approximately to a pulse duration (time t) of 1 s and an intensity (I) of 50 W/cm^2. For t greater than 1 s the data represented by Curve A lies above the dashed line, and for t less than 1 s the reverse is true. Thus Statement B does not apply to the Fry-Dunn data on mouse paralysis at 37° C for $t < 1$ s. However, in similar experiments by the same authors at lower temperatures (2°, 10° and 20° C) the product (It) corresponding to "threshold" for paralysis was always greater than 50 J/cm^2; for these conditions both Statements apply.

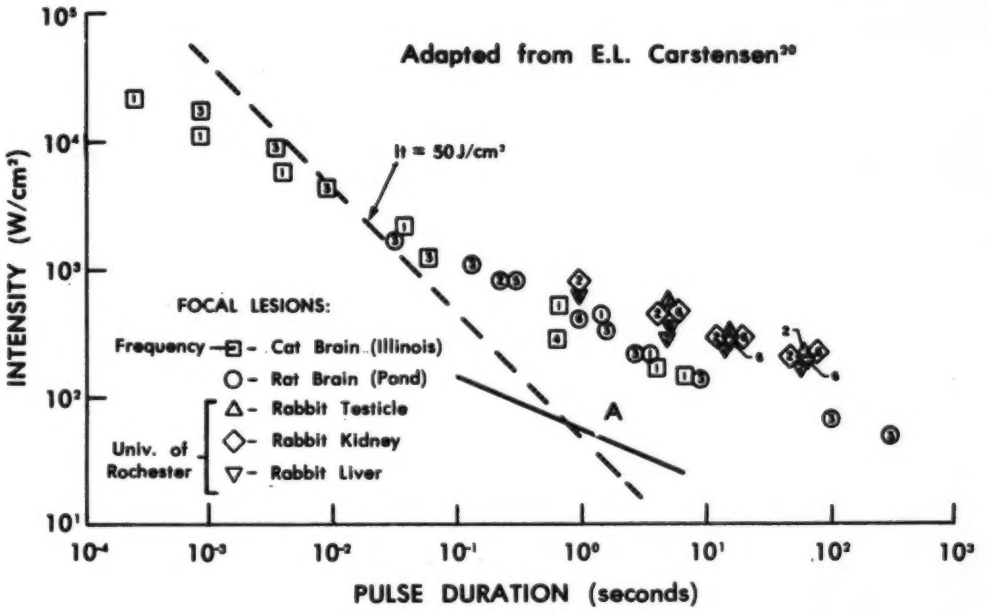

Figure 16. Intensity levels at which lesions are produced by focussed ultrasound in mammalian tissues. From E.L. Carstensen, Data presented at the 20th Annual Meeting of the American Institute for Ultrasound in Medicine, Winston-Salem, N.C., October 4-9, 1975. Curve A is for data of Fry and Dunn on paralysis in young mice at 37°C. See text.

For more than two decades there have been investigations on conditions for producing focal lesions in mammalian brain and other organs. Such a lesion is a localized region of damage, identified by histological methods, generated by focused megahertz ultrasound in a cigar-shaped volume of sub-millimeter lateral dimensions. Generated by single pulses of durations extending from hundreds of seconds down to fractions of milliseconds, they have been studied by groups at the University of Illinois, Massachusetts Institute of Technology, Guy's Hospital (London, England) and the University of Rochester. Results have been summarized by Fry, Kossoff, Eggleton and Dunn (98), and by Carstensen (99), and are shown on figure 16. In this figure the intensity (I) plotted is a threshold value for observation of a histological lesion. We see that the threshold value of (It) is equal to 50 J/cm^2 when t is about 0.02 s, corresponding to an intensity I of 2500 W/cm^2. For $t > 0.02$ s the product (It) is larger and for $t < 0.02$ s it is smaller, reaching about 5 J/cm^2 at 1 MHz for t = 0.002 s. The thresholds for focal lesions in rabbit testicle, kidney and liver are relatively high. For pulses of 1-100 sec duration the threshold values of (It) are 500 J/cm^2 and higher.

5.3 LEVELS GENERATED BY COMMERCIAL DEVICES

For comparison with the intensity levels at which bioeffects occur, we now consider briefly the levels which characterize the output of commercial diagnostic instruments which are in use. Representative results are shown in figure 17. These data come primarily from Carson (100), based on measurements made at the University of Colorado Medical Center; several results are from Rooney (101), obtained at the University of Vermont. (The data in figure 17 are primarily for equipment used in the U.S.A. Similar information on equipment in

the U.K. have been published by HIll (102)). A variety of indices have been used for the output of commercial devices. For continuous (CW Doppler) systems a common one is a spatially averaged (Sa) value at the transducer face. Measuring the total power output in watts and dividing by the effective transducer area in square centimeters yields the Sa intensity in watts/cm^2. This is the quantity represented by open circles in figure 17 for continuous systems. For these systems the peak (Sp) intensity in the field typically occurs on the axis of the transducer and several centimeters from its front face; the Sp intensity is greater than the Sa value by a factor of, say 2 to 4, as discussed in Section 5.2. For the CW devices represented here the Sa intensities vary from 0.001 to 0.30 W/cm^2. The Sp values are represented for two units by filled circles, and are at the levels of 0.005 and 0.5 W/cm^2. It is this Sp intensity which is represented in figure 15 by each datum point for a bioeffect caused by continuous ultrasound.

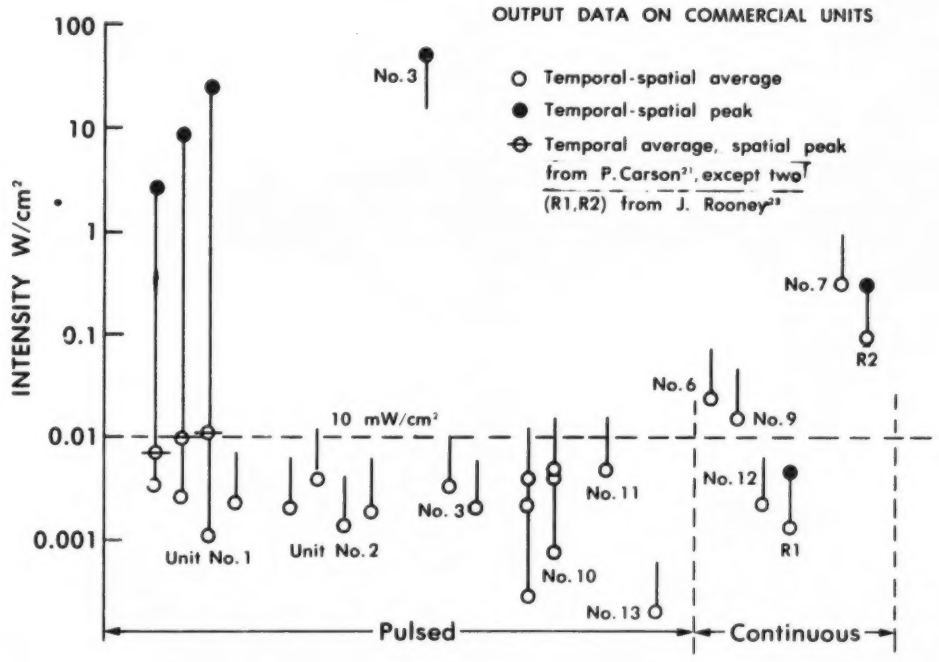

Figure 17 . Representative data on intensity levels generated by commercial instruments. From P. Carson (Statement to FDA Classification Panel on Ophthalmic Devices, October 1975) and J.A. Rooney (Ultrasound in Med. and Biol. 1, 13-16 (1973).

For pulsed devices the quantity often quoted, and represented here by an open circle, is the SaTa value, i.e., a value averaged both with respect to space and time. As stated earlier the time-averaged (Ta) intensity (whether space averaged, or evaluated at a given point) during a series of pulses is equal to the product of the duty factor and the corresponding temporal peak (Tp) intensity, the latter being the intensity which exists during a pulse. Since the duty factor is usually small, Tp intensities are typically much larger than the Ta values. The filled circles in figure 17 represent SpTp intensities, i.e., they are peak values with respect to both space and time. For the commercial devices on which Carson made measurements we see from figure 17 that the SpTp values ranged from 3 W/cm^2 to 80 W/cm^2. For several devices the spatial-

peak, time-averaged (SpTa) intensity was also determined by Carson; each of these is represented on figure 17 by an open circle cut by a line segment (θ). It is this SpTa intensity which is plotted as ordinate in figure 15.

Judging by the three devices for which SpTa intensities are quoted by Carson, existing pulsed equipment generates fields in which these intensities do not much exceed 10 mW/cm^2. Of the six CW units for which data are given by Carson and Rooney, the units numbered 6, 9, 12 and R1 are used in fetal diagnosis. For two of these (12 and R1) the Sp intensity apparently lies below 10 mW/cm^2 while for the other two (6 and 9) the intensity extends well above this value. The units 7 and 12 generate much higher levels, and are for peripheral vascular diagnosis.

From these data we see that 10 mW/cm^2 exceeds the SpTa intensity values for some pulsed equipment and also the Sp intensity values for some of the CW devices. For those devices the intensity is thus less by at least a factor of ten than the lowest intensity at which bioeffects have been observed in mammalian tissues, according to Statement A.

5.4 MECHANISMS FOR EFFECTS ON MAMMALIAN TISSUES AT LOW INTENSITY LEVELS

For most of the data represented in figure 15 it is not possible to answer the question "Why did the effect occur?" An exception, of course, is point F, representing conditions for production of fetal abnormalities. It follows from the way this point was arrived at (see Section 5.2) that sonically generated temperature elevation is the cause of these abnormalities. In connection with the datum point C representing alterations in wound-healing processes, the authors gave evidence of a nonthermal mechanism: a comparison of treatments with pulsed ultrasound and CW ultrasound showed increased tissue response to the former, although the temperature elevation was believed the same for both. (See Section 5.2.)

We now explain the origin of the solid curve shown in figure 15. This is a computed curve which becomes identical with the dashed line "It = 50 J/cm^2" at small values of t and approaches the constant intensity 100 mW/cm^2 for large t. It applies to a simple model and represents combinations of intensity and time which lead to a given temperature elevation. More specifically, the curve is based on the theory for heat generation and conduction taken up in Section 2.4 and is closely related to those plotted in figure 4. Heat is assumed generated by a plane ultrasonic wave in a sphere of radius 1 cm and absorption coefficient 0.1 Np/cm; the latter is an intermediate value, typical of brain and liver at a frequency of 1 MHz. (See Table 1, Section 2.1.) The surrounding medium is assumed free of absorption, while the heat conductivity coefficient and heat capacity are assumed the same as for water both inside and outside the sphere. The solid curve represents combinations of intensity and time which lead to the temperature elevation 2.4° C at the center of the sphere.

Evidently the curve based on this simple idea serves well in figure 15 as a boundary between regions representing conditions where bioeffects do and do not occur. Only point D lies below this curve, and this represents observations which have proved difficult to repeat. It is as if the criterion for a reproducible bioeffect were that a temperature rise of 2.4° C should be attained in a sphere of 1 cm radius, in which thermal properties are like those for water, but in which the attenuation coefficient is 0.1 Np/cm.

However, it must be emphasized that this simple model is not proposed seriously as a means of explaining the various bioeffects represented in figure 15. A number of important experimental facts have been ignored, including the

evidence mentioned earlier that the results represented by point C did not arise from temperature elevation. The solid curve in figure 15 is best regarded as the result of a procedure for fitting the data. It is puzzling that the curve serves this purpose as well as it does.

5.5 MINIMUM LEVELS WHEN CAVITATION IS A MECHANISM

As stated in Section 3, when bioeffects are obtained in suspension under in vitro conditions, and temperature elevation is not a factor, the mechanism is usually some form of cavitation activity. It is often found that cavitation does not occur in an ultrasonic field unless a certain threshold intensity is exceeded. Typically the value of this threshold depends on the state of nucleation, i.e., on the number, size and distribution of small gaseous or vaporous bodies. Since nucleation often depends on accidental circumstances, such as the presence of gas-trapping impurity particles or the existence of small cracks or crevices in container walls, it is understandable that the threshold for cavitation varies greatly from one circumstance to another. Also, it is not surprising that the threshold for cavitation-dependent bioeffects is variable.

There is not space here, nor is there need, for more than a brief discussion of observed cavitation-dependent biophenomena in solution; the subject has been reviewed in References 1, 2 and 22. Under some circumstances, especially when focused megahertz ultrasound is used, changes in suspensions of cells or macromolecules occur only at intensities of hundreds of watts per square centimeter or more. However, using other arrangements, bioeffects are observed in suspension at levels of only a few watts per square centimeter. By using a special arrangement which favored nucleation Clarke and Hill (103) observed cellular changes at intensities as low as 1.5 W/cm^2. Among the lowest intensity levels which have so far been reliably reported for significant bioeffects in solution are those for the work of Williams and O'Brien (104) showing altered recalcification time of platelet-rich plasma; these investigators observed changes after application of ultrasound for five minutes at spatial peak (Sp) levels as low as 0.18 W/cm^2. Another report of cellular changes at low intensities is that of Lock, Fischer and Kuwert (105); they found reduction in cell proliferation in cultures subjected to 2.5 MHz ultrasound for 10 min at an Sp intensity of about 0.3 W/cm^2. (We estimated the Sp intensity from a cited Sa value of 0.1 W/cm^2.)

Still another low-level effect was observed by Harvey, Dyson, Pond and Grahame (106). Using 1 MHz or 3 MHz ultrasound in 2 ms pulses with 10 ms spacing, they found alterations in cultures of human fibroblasts at SpTa intensities as low as about 80 mW/cm^2 applied for 5 min. These changes include (a) reduction of cell numbers, (b) reduced ability of cells to attach (to culture dish), (c) damage to intracellular structures, and (d) increase in rate of protein synthesis. The authors suggest that the increased rate of synthesis may be an indirect result of damage to lysosomes. In experiments with CW sonation the authors find less biological effect, for a given SpTa intensity, than for their pulsed regime.

Considering all presently known results on bioeffects obtained in suspension under in vitro conditions at megahertz frequencies, it appears that none have been reported at intensity levels much less than about 80 mW/cm^2 and hence as far as existing data are concerned, Statement A applies approximately even to these. We recall, however, the calculations in Section 4.5 showing theoretical possibilities for cellular changes produced by stable cavitation at very low intensity levels. (Also, subsequent to the talks on which this report is based, a short note has been published, in which the authors report altered uptake of

tritiated thymidine in hela cells by pulsed ultrasound of unusually low levels (107)).

Whatever the situation may prove to be for Statement A, it appears that Statement B is unlikely to be useful for cavitation-dependent bio-changes. It is to be expected that the time required for such changes to be observable will be very dependent on the initial state of cavitation nucleation. If gas bubbles of effective size are present initially the sonic action may begin promptly; if time must be allowed for them to grow there may be a significant delay. When such delays exist it is to be expected that the results obtained from repeated-pulse regimes will depend on the pulsing parameters. We have already seen this to be true in the experiments of Hill and co-workers (36). (See Section 3.10.) In their experiments effective cavitation action was obtained with pulses 10 ms in duration, but very little with pulses as short as 1 ms.

In experiments with pulsed ultrasound we often do not know the minimum number say, N_{min}, of pulses required to produce an observable result. Suppose each pulse produces a fixed "amount of bioeffect", where the "amount" might represent, for example, (a) the number of molecules altered in a chemical reaction or (b) the number of cells affected. Then N_{min} would depend on the sensitivity of the method used to determine the change. With a very sensitive method we might have $N_{min} = 1$; then for the product (It) referred to in Statement B, the intensity I is just the spatial-peak value which appears during a pulse and t is the duration of a single pulse. If I is in the range of 1-10 W/cm^2 (as was true in the experiments of Hill, et al. (36)) and t is only 10 ms we see that (It) is indeed very small, being equal to or less than 0.1 J/cm^2. Obviously Statement B is invalid for such a situation; it applies only if N_{min} is rather large.

We have already considered evidence that cavitation occurs in plant tissues. By making observations through a microscope while ultrasound of 1 MHz frequency is applied, information has been obtained on the levels required to produce visible displacements and deformations of intracellular structures (46). Slight movements of individual particles are occasionally seen at levels equivalent to a plane wave (spatial peak) intensity of about 35 mW/cm^2 but they are seen more regularly at 350 mW/cm^2. At these low levels the effects usually occur only near gas-filled intercellular spaces in that part of the sound field where the pressure amplitude is maximum. Evidence has not been obtained on the importance to the tissue of this disturbance, and it is therefore not possible to state whether the observed effects at 35 mW/cm^2 should be regarded as an exception to Statement A.

However, it is rather definite that Statement B is invalid for plant tissues. In direct observations through the microscope irreversible damaging effects are observed at 3.5 W/cm^2 after ultrasound has been applied for a few seconds (46); hence the product (It) corresponding to a significant bioeffect is less than 50 J/cm^2. In recent work at the University of Rochester, Miller and co-workers (108) report reduction in mitotic index for pea root at intensity-time products of 20-30 J/cm^2 or less. While the mechanisms for this result are not known, it may possibly be a consequence of stable cavitation occuring in the plant tissues. If so, the effects in plants give further support to the idea that cavitation-dependent phenomena may violate Statement B.

At present there is insufficient published evidence on which to form a judgment as to whether ultrasonic cavitation activity is significant in mammalian tissues at low intensity levels. When very high intensity levels, about 1500 W/cm^2, are generated by focused ultrasound in brain tissue, gross tissue damage is produced even for exposure times of the order of milliseconds (Fry, Kossoff, Eggleton and Dunn (42)). This damage, differing in character from that observed

in lower levels, is apparently caused by cavitation of a rather violent nature. It is interesting that the conditions required for violent cavitation at the focus in brain are about the same as the (large I, small t) conditions seen in figure 16, for which the (It) threshold is less than 50 W/cm^2.

6. CONCLUSIONS

In this report an attempt has been made to take up physical topics which give insight on interactions between ultrasound and biological systems. In addition we have taken up examples of biological changes which illustrate the interactions. Some of these examples are important for indicating the lowest intensity levels at which bioeffects of ultrasound have been observed. Others help to indicate the extent to which bioeffects can be understood in terms of physical mechanisms.

Some situations where the sound acts via a thermal mechanism have proved amenable to detailed analysis. For example, Pond (109), Robinson and Lele (110) and Dunn, Lohnes and Fry (77) have shown that under a wide range of conditions the intensity thresholds for focal lesions in brain can be calculated very well on a thermal hypothesis. In the present report, a thermal model was found useful for "curve-fitting" purposes. From mathematical expressions in Section 2 we plotted the intensity I required to yield a given temperature rise at the center of an absorbing sphere after a given sonication time t. We found that such a plot of I versus t, when suitable parameters were chosen for the sphere, served rather well as a lower bound to the bioeffects data in figure 15. This finding is interesting and convenient, but it should not be taken too seriously, since the absorbing-sphere model is surely a crude one for the various systems to which figure 15 applies.

When cavitation is the mechanism the situation is more complex. At the low intensity levels characteristic of medical ultrasound it would seem that stable cavitation is a more probable mode than transient cavitation. For either mode the minimum values of intensity I and time t for producing an observable biological effect will depend on the initial state of nucleation. If gas bubbles of effective size are present initially, the required values of I and t may be relatively small. This is indicated, for example, by a speculative calculation for cell suspensions in Section 4.5. It is also consistent with observations on plant tissues: for this tissue is typically permeated wih gas-filled channels, and it is therefore reasonable that only small values of (It) are required for observable effects on these tissues, as noted in Section 5.5. It is also to be expected for cavitation-dependent phenomena that repeated pulses of ultrasound will be more effective than continuous operation, for a given time-averaged intensity. At present little is known about the extent to which gas-filled spaces may be present in mammalian tissues. Correspondingly we have little basis for judging whether stable cavitation occurs, or is significant at low intensity levels in such tissues.

Much less is known about biological effects generated by ultrasound in the absence of either cavitation or temperature elevation. It is known that radiation forces and torques can cause relocation, distortion, reorientation and/or spinning of intracellular particles, of whole cells, and of larger bodies. Also interparticle radiation forces can produce aggregation of cells or other units (Sections 4.6 and 4.7). However, as yet there does not seem to have been any instance in which radiation force or torque was identified specifically as the mechanism for a significant bioeffect at megahertz frequencies.

Another nonthermal characteristic of ultrasound which has biological significance is acoustic microstreaming, a phenomenon which can occur in the

absence of cavitation (although it often occurs as a concomitant of stable cavitation). In vitro experiments at low ultrasonic frequencies have shown that viscous stresses associated with this circulatory motion can cause damage to cells in suspension (Section 4). Also, applying localized 20 kHz ultrasound to an intact femoral blood vessel in a young adult mouse Williams (111) has demonstrated acoustic microstreaming in vivo, and has shown that it can lead to clot formation. At megahertz frequencies microstreaming is seen in plant tissues under some circumstances (45,46), but little is known about its signficance for mammalian tissues.

In an overall view of our present state of knowledge, it seems to this author that we have some understanding of physical mechanisms applicable to liquids and suspensions. However, the task has barely begun of determining mechanisms for biological effects in mammalian tissues. The only detailed explanations that appear to have been successful so far are those (77, 109, 110) for the thermal origin of focal lesions in brain, and those for the thermal origin of fetal abnormalities (91). While the phenomenon of red-cell stasis in the circulation (66) appears to be a consequence of radiation pressure, a satisfactory quantitative theory for it has apparently not yet been developed.

That there are many unanswered questions in regard to bioeffects is evidenced by the following partial list of phenomena caused by ultrasound, for which explanations are not known (112):

Decrease in the number of glycogen granules in liver and muscle.

Swelling and/or disruption of mitochondria in liver, muscle and kidney.

Destruction of lysosomes in liver.

Alteration in transport across membranes.

Ultrastructural changes in muscle.

Changes in cell division processes.

Structural changes within the inner ear.

These phenomena occur in cytoplasmic and membranous media about whose acoustic properties little is known. We have essentially no information on nonthermal means for ultrasonic action on such media, analogous to the mechanisms of cavitation, radiation force and acoustic streaming which apply to liquids and suspensions. To represent living substance a possible model medium might be a soft solid such as an aqueous gel. It seems likely that research on interactions between ultrasound and well-chosen simple solid-like media would give insight on interactions between ultrasound and biomedia. See H.M. Frost (81).

Investigation of mechanisms not only leads to a coherent structure of knowledge which is intellectually satisfying. It is also of considerable practical importance. We have seen that even now it is sometimes possible to predict conditions under which bioeffects occur, once the mechanism is known. As we develop our understanding we can expect further improvement in our ability to predict, and hence also in our ability to choose conditions for effective and wise use of ultrasound in medicine.

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